(defined in Example 59), containing an H6 promoted truncated HIV-1 **envelope** gene, into the filled-in PamHI site of pHIVG7. The plasmid generated by this manipulation is; called pHIVGE12. pHIVGE12 was used. . .

DETD The H6-promoted HIV-1(MN) **envelope** (gp120) gene was then inserted into pHIVGE14. This was accomplished by cloning the oligonucleotides, HIVL29 (SEQ ID NO:421) (5'-GGCCGCAAC-3') and. . .

DETD The H6-promoted **envelope** (g)p120) gene and the I3L-promoted gag and pol genes were then inserted into a vaccinia virus insertion vector. This was. . .

DETD . . . by the gpl60 gene. This was accomplished by cloning the 2,600 bp NruI-NotI fragment of pH6HMNE, containing the entire HIV-1(MN) **envelope** (gp160) gene, into the 8,000 bp partial NruI-LotI fragment of pHIVGE16. The plasm.id generated by this manipulation is called pHIVGE19.

DETD . . . gene products. CEF cell monolayers were either mock infected, infected with the parental virus or infected with vCP117 at an **m**.o.i. of 10 PFU/cell. Following an hour adsorption period, the inoculum was aspirated and the cells were overlayed with 2 mls. . .

DETD . . . the gpl60 gene. This was accomplished by cloning the 2,600 bp NruI-NotI fragment of pH6HMNE, containing the entire HIV1 (MN) **envelope** (gp160) gene, into the 9,800 bp NruI-NotI fragment of pHIVGE15. The plasmid generated by this manipulation is called pHIVGE18.

DETD . . . gene products. CEF cell monolayers were either mock infected, infected with the parental virus or infected with VCP130 at an **m**.o.i. of 10 PFU/cell. Following an hour adsorption period, the inoculum was aspirated and the cells were overlayed with 2 mls. . .

DETD . . . qaq-pol and env genes would also produce such particles. Furthermore, if these ALVAC-based recombinants were used to infect non-avian cells (i.**e**. Vero, MRC-5, etc.) then HIV-1 virus-like particles could be purified without any poxvirus virion contaminants.

DETD . . . evaluate particle formation using Vero cells infected with vCP156, the following experiment was performed. Vero cells were infected at an **m**.o.i. of approximately 5 pfu/cell. After a 24 hr infection period, the supernatant was harvested and clarified by centrifugation at 2000. . . With the size exclusion noted above, the p24 would have passed through unless it was in a higher structural configuration (i.**e**. virus-like particles). Therefore, these results strongly suggest that HIV-1 virus-like particles containing the gp120 **envelope** component are produced in vCP156 infected cells.

DETD . . . peptides include! the 51 amino acid N-terminal portion of HIV-1 (IIIB) env, residues 1-50 (plus initiating Met) based on Ratner **e**!t al. (1985). The amino acid sequence of this signal region (SEQ ID NO:443) is MKEQKTVAMRVKEKYQHLWRWGWRWGTMLLGMIMICSATEKLWVTVYYGVP. This is followed by the. . .

DETD . . . gene products. Vero cell monolayers were either mock infected, infected with the parental virus or infected with vP1045 at an **m**.o.i. of 10 PFU/cell. Following an hour adsorption period, the inoculum was aspirated and the ce:Lls were overlayed with 2 mls. . .

DETD . . . gene products. CEF cell monolayers were either mock infected, infected with the parental virus or infected with VCP153 at an **m**.o.i. of 10 PFU/cell. Following an hour adsorption period, the inoculum was aspirated and the cells were overlayed with 2 mls. . .

DETD . . . precursor proteins. Vero cell monolayers were either mock infected, infected with the parental virus or infected with vP948 at an **m**.o.i. of 10 PFU/cell. Following an hour adsorption period, the inoculum was aspirated and the cells were overlayed with 2 mls. . .

DETD Macague sera from SIV seropositive individuals specifically precipitated the SIV cac precursor protein and the **envelope** glycoprotein from vP948 infected cells, but did not precipitate SIV-specific proteins from mock infected cells.

DETD The plasmid, pSIVEMVC, contains the H6-promoted SIV_{MAC142} **envelope** gene (in vitro selected truncated version). The region of the **envelope** gene containing the premature termination codon was cloned into pBSK+. This was accomplished by cloning the 1,120 bp ClaI- amHI. .

DETD At day 56 (i.e. 28 days after the second injection) protective titers were achieved in 0/3 of Group A, 2/3 of Group B and. . .

DETD . . . 35 56

1	$10^3.5$					
		<0.1	<0.1	<0.1	<0.1	0.2
3	$10^3.5$					
		<0.1	<0.1	<0.1	<0.1	<0.1
4	$10^3.5$					
		<0.1	<0.1	<0.1	<0.1	<0.1
	G.M.T.	<0.1	<0.1	<0.1	<0.1	<0.1
6	$10^4.5$					
		<0.1	<0.1	<0.1	<0.1	<0.1
7	$10^4.5$					
		<0.1	<0.1	<0.1	2.4	1.9
10	$10^4.5$					
		<0.1	<0.1	<0.1	1.6	1.1
	G.M.T.	<0.1	<0.1	0.1	0.58	0.47
11	$10^5.5$					
		<0.1	<0.1	1.0	3.2	4.3
13	$10^5.5$					
		<0.1	<0.1	0.3	6.0	8.8
14	$10^5.5$					
		<0.1	<0.1.	. .	0.3	3.7
21	$10^5.5$					
		<0.1	<0.1	0.2	2.6	3.9
23	$10^5.5$					
		<0.1	<0.1	<0.1	1.7	4.2
25	$10^5.5$					
		<0.1	<0.1	<0.1	0.6	0.9
	G.M.T.	<0.1	<0.1	0.16	1.9	4.4*
2	HDC	<0.1	<0.1	0.8	7.1	7.2
5	HDC	<0.1	<0.1	9.9	12.8	18.7
8	HDC	<0.1	<0.1.	. .	7.7	20.7
19	HDC	<0.1	<0.1	2.6	9.9	9.1
22	HDC	<0.1	<0.1	1.4	8.6	6.6
24	HDC	<0.1	<0.1	0.8	5.8	4.7
	G.M.T.	<0.1	<0.1	2.96	9.0	11.5*

*p = 0.007 student t test

DETD Using NYVAC-JEV recombinants, protection against Japanese **Encephalitis virus** was provided. NYVAC vP866, NYVAC recombinants vP908 and vP923, and vaccinia recombinants vP555 and vP829 were produced as described herein.

DETD . . . positioned behind the early/late H6 promoter. Recombinant vP908 (and vP555; Mason et al., 1991) includes the putative 15 amino acid **signal sequence** preceding the N-terminus of **prM**, **prM**, **E**, NS1 and NS2A. Recombinant vP923 (and vP828; Konishi et al., 1991) encodes the putative **signal sequence** of **prM**, **prM**, and **E**.

DETD Synthesis of **E** and NS1 bv Recombinant Vaccinia Viruses.

DETD Immunoprecipitation of the **E** or NS1 gene was performed using a monoclonal antibody specific for **E** or NS1. Proteins reactive with the **E** MAb were synthesized in cells infected with vP555, vP908 and vP923, and proteins reactive with NS1 MAb were synthesized in. . . cells infected with vP555 and vP908 but not in cells infected with vP?923. vP555 infected cells produced correct forms of **E** and NS1 inside and outside of the cell. The proteins produced by vP908 and vP923 were identical in size to those produced by vP555. For both **E** and NS1, the extracellular forms migrated slower than the intracellular forms in SDS-PAGE, consistent with maturation of the N-linked glycans. . . the JEV genome (Mason et al., 1987). Immunoprecipitates prepared from radiolabeled vaccinia recombinant infected cells using a MAb specific for **M** (and **prM**) revealed that **prM** was synthesized in cells infected with vP908 and vP923.

DETD The immune response to **E** correlated well with the results of the NEUT

and HAI tests. The RIP response to **E** observed in swine immunized with vP923 on day 35 was higher than the RIP response to **E** in swine immunized with vP908, whereas the HAI titers on day 35 were equivalent in the two groups. However, NEUT. . . be induced but the quantitative aspects of the RIP analysis was not further validated. Weak RIP responses of sera to **E** on day seven in spite of relatively high NEUT antibody titers could be explained by IgM antibody early after immunization.. . .

DETD . . . sera collected 20 days post-challenge for antibodies against JEV. The swine vaccinated with vP908 or vP923 had higher responses to **E** than those inoculated with PBS or vP866, indicating that the antibody reactivity to **E** that was present before challenge was boosted by JEV infection. Reactions to NS3 and NS5, JEV proteins which were not. . .

DETD TABLE 52

Immunization and JEV challenge in mice

Immunizing Virus[a]	JEV Genes Expressed	Antibody titer NEUT[b]	HAI[c]	Survival[d]
vP829	**prM, E**	1:320	1:80	10/10 (100%)
vP866	None	<1:10	<1:10	0/12 (0%)
vP908	**prM, E,** NS	1:320	1:80	11/12 (92%)
vP923	**prM, E**	1:320	1:80	12/12 (100%)

[a] Vaccinia recombinant virus used for immunizing groups of 4week old mice.

[b] Serum dilution yielding. . .

DETD . . . recombinants have also been showe to elicit measles virus neutralizing antibodies in rabbits and protection against pseudorabies virus and Japanese **encephalitis virus** challenge in swine. The highly attenuated NYVAC strain confers safety advantages with human and veterinary applications (Tartaglia et al., 1990).. . .

DETD . . . skin); c) absence of testicular inflammation (nude mice); d) greatly reduced virulence (intracranial challenge, both three-week old and newborn mice); **e**) greatly reduced pathogenicity and failure to disseminate in immunodeficient subjects (nude and cyclophosphamide treated mice); and f) dramatically reduced ability. . .

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L14 ANSWER 11 OF 15 USPATFULL on STN

1998:57530 Alvac canarypox virus recombinants comprising heterlogous inserts.

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APPLICATION: US 1995-457007 19950601 (8) <--
DOCUMENT TYPE: Utility; Granted.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB What is described is a modified vector, such as a recombinant poxvirus, particularly recombinant vaccinia virus, having enhanced safety. The modified recombinant virus has nonessential virus-encoded genetic functions inactivated therein so that virus has attenuated virulence. In one embodiment, the genetic functions are inactivated by deleting an open reading frame encoding a virulence factor. In another embodiment, the genetic functions are inactivated by insertional inactivation of an open reading frame encoding a virulence factor. What is also described is a vaccine containing the modified recombinant virus having nonessential virus-encoded genetic functions inactivated therein so that the vaccine has an increased level of safety compared to known recombinant virus vaccines.

CLM What is claimed is:
1. An attenuated virus having all the identifying characteristics of: an ALVAC canarypox virus.

2. A virus which is ALVAC.

3. A vector which comprises the virus of claim 1.

4. A vector which comprises the virus of claim 2.

5. A virus as claimed in claim 2 further comprising exogenous DNA from a non-poxvirus source in a nonessential region of the virus genome.

6. A virus as claimed in claim 5 wherein the exogenous DNA is selected from the group consisting of rabies virus, Hepatitis B virus, Japanese **encephalitis virus, yellow fever virus, Dengue** virus, measles virus, pseudorabies virus, Epstein-Barr virus, herpes simplex virus, human immunodeficiency virus, simian immunodeficiency virus, equine herpes virus, bovine herpes virus, bovine viral diarrhea virus, human cytomegalovirus, canine parvovirus, equine influenza virus, feline leukemia virus, feline herpes virus, Hantaan virus, C. tetani, avian influenza virus, mumps virus and Newcastle Disease virus.

7. A virus as claimed in claim 6 wherein the virus is a canarypox virus, the non-poxvirus source is rabies virus, and the canarypox virus is vCP65 or vCP136.

8. A virus as claimed in claim 6 wherein the virus is a canarypox virus, the non-poxvirus source is human immunodeficiency virus and the canarypox virus is vCP95, vCP112, vCP60, vCP61, vCP125, vCP124, vCP126, vCP144, vCP120, vCP138, vCP117, vCP130, vCP152, vCP155, vCP156, vCP146, vCP148, vCP154, vCP168 or vCP153.

9. A virus as claimed in claim 6 wherein the virus is a canarypox virus, the non-poxvirus source is equine herpes virus and the canarypox virus is vCP132.

10. A virus as claimed in claim 6 wherein the virus is a canarypox virus, the non-poxvirus source is human cytomegalovirus and the canarypox virus is vCP139.

11. A virus as claimed in claim 6 wherein the virus is a canarypox virus, the non-poxvirus source is canine parvovirus and the canarypox virus is vCP123 or vCP136.

12. A virus as claimed in claim 6 wherein the virus is a canarypox virus, the non-poxvirus source is Epstein-Barr virus and the canarypox virus is vCP167.

13. A virus as claimed in claim 6 wherein the virus is a canarypox virus, the non-poxvirus source is equine influenza virus and the canarypox virus is vCP128 or vCP159.

14. A virus as claimed in claim 6 wherein the virus is a canarypox virus, the non-poxvirus source is feline leukemia virus and the canarypox virus is vCP177, vCP83, vCP35, vCP37, vCP87, vCP93 or vCP97.

15. A virus as claimed in claim 6 wherein the virus is a canarypox virus, the non-poxvirus source is feline herpes virus and the canarypox virus is vCP162.

16. A virus as claimed in claim 6 wherein the virus is a canarypox virus, the non-poxvirus source is Hantaan virus and the canarypox virus is vCP114 or vCP119.

17. A virus as claimed in claim 6 wherein the virus is a canarypox virus, the non-poxvirus source is Hepatitis B and the canarypox virus is vCP169 or vCP157.

18. A virus as claimed in claim 6 wherein the virus is a canarypox virus, the non-poxvirus source is C. tetani and the canarypox virus is vCP161.

19. A virus as claimed in claim 6 wherein the virus is a canarypox virus, the non-poxvirus source is mumps virus and the canarypox virus is vCP171.

20. A virus as claimed in claim 6 wherein the virus is a canarypox virus, the non-poxvirus source is Japanese **encephalitis virus** and the canarypox virus is vCP107 or vCP140.

21. A virus as claimed in claim 6 wherein the virus is a canarypox virus, the non-poxvirus source is simian immunodeficiency virus, and the canarypox virus is vCP172.

22. A virus as claimed in claim 6 which is a rabies virus recombinant canarypox virus which is vCP65.

23. A virus as claimed in claim 6 which is a human immunodeficiency virus recombinant canarypox virus which is vCP95, vCP112, vCP60 or vCP61.

24. A virus as claimed in claim 1 further comprising exogenous DNA from a non-poxvirus source in a non-essential region of the virus genome.

25. An immunological composition for inducing an immunological response in a host animal inoculated with said composition, said composition comprising the virus of any one of claims 1, 2 or 5 to 24, or, a vector as claimed in claim 3 or 4, and a carrier.

26. The immunological composition of claim 25 which is a vaccine.

27. A method of expressing a gene product in a cell cultured in vitro comprising introducing into the cell a virus as claimed in any one of claims 1, 2 or 5 to 24, or, a vector, transforming cell with the expression vector, cultivating the transformed cell under conditions which allow expression of the recombinant poxvirus, and further purifying the protein as claimed in claim 3 or 4.

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SUMM . . . sequence to be inserted into the virus, particularly an open reading frame from a non-pox source, is placed into an **E.** coli plasmid construct into which DNA homologous to a section of DNA of the poxvirus has been inserted. Separately, the. . . flanking a region of pox DNA containing a nonessential locus. The resulting plasmid construct is then amplified by growth within **E.** coli bacteria (Clewell, 1972) and isolated (Clewell et al., 1969; Maniatis et al., 1982).

SUMM Second, the isolated plasmid containing the DNA gene sequence to be inserted is transfected into a cell culture, **e.**g. chick embryo fibroblasts, along with the poxvirus. Recombination between homologous pox DNA in the plasmid and the viral genome respectively. . .

SUMM Cowpox virus (Brighton red strain) produces red (hemorrhagic) pocks on the chorioallantoic **membrane** of chicken eggs. Spontaneous deletions within the cowpox genome generate mutants which produce white pocks (Pickup et al., 1984). The. . .

DRWD FIG. 26 shows the nucleotide sequence of FeLV-B **Envelope** Gene (SEQ ID NO:310);

DETD . . . from Bethesda Research Laboratories, Gaithersburg, Md., New England Biolabs, Beverly, Mass.; and Boehringer Mannheim Biochemicals, Indianapolis, Ind. Klenow fragment of **E.** coli polymerase was obtained from Boehringer Mannheim Biochemicals. BAL-31 exonuclease and phage T4 DNA ligase were obtained from New England. . .

DETD . . . NcoI site (pos. 172,253) were removed by digestion of pSD419 with NcoI/SmaI followed by blunt ending with Klenow fragment of **E.** coli polymerase and ligation generating plasmid pSD476. A vaccinia right

flanking arm was obtained by digestion of pSD422 with HpaI. . . ID NO:6/SEQ ID NO:7) ##STR3## generating pSD479. pSD479 contains an initiation codon (underlined) followed by a BamHI site. To place **E**. coli Beta-galactosidase in the B13-B14 (u) deletion locus under the control of the u promoter, a 3.2 kb BamHI fragment. . .

DETD . . . at the pUC/vaccinia junction was destroyed by digestion of pSD478 with EcoRI followed by blunt ending with Klenow fragment of **E**. coli polymerase and ligation, generating plasmid pSD478E.sup.-. pSD478E.sup.- was digested with BamHI and HpaI and ligated with annealed synthetic oligonucleotides. . .

DETD . . . XbaI within vaccinia sequences (pos. 137,079) and with HindIII at the pUC/vaccinia junction, then blunt ended with Klenow fragment of **E**. coli polymerase and ligated, resulting in plasmid pSD483. To remove unwanted vaccinia DNA sequences to the right of the A26L. . . digestion with BglII (pos. 140,136) and with EcoRI at the pUC/vaccinia junction, followed by blunt ending with Klenow fragment of **E**. coli polymerase and ligation. The resulting plasmid was designated pSD489. The 1.8 kb ClaI (pos. 137,198)/EcoRV (pos. 139,048) fragment from. . .

DETD A 3.3 kb BglII cassette containing the **E**. coli Beta-galactosidase gene (Shapira et al., 1983) under the control of the vaccinia 11 kDa promoter (Bertholet et al., 1985;. . .

DETD A 3.2 kb BglII/BamHI (partial) cassette containing the **E**. coli Beta-galactosidase gene (Shapira et al., 1983) under the control of the vaccinia 11 kDa promoter (Bertholet et al., 1985;. . .

DETD . . . were removed from the pUC/vaccinia junction by digestion of pSD466 with EcoRI/BamHI followed by blunt ending with Klenow fragment of **E**. coli polymerase and ligation. Recombination between vP708 and pSD467 resulted in recombinant vaccinia deletion mutant, vP723, which was isolated as. . .

DETD . . . SphI and religated, forming pSD451. In pSD451, DNA sequences to the left of the SphI site (pos. 27,416) in HindIII **M** are removed (Perkus et al., 1990). pSD409 is HindIII **M** cloned into pUC8.

DETD To provide a substrate for the deletion of the [C7L-K1L] gene cluster from vaccinia, **E**. coli Beta-galactosidase was first inserted into the vaccinia M2L deletion locus (Guo et al., 1990) as follows. To eliminate the. . . unique BglII site inserted into the M2L deletion locus as indicated above. A 3.2 kb BamHI (partial)/BglII cassette containing the **E**. coli Beta-galactosidase gene (Shapira et al., 1983) under the control of the 11 kDa promoter (Bertholet et al., 1985) was. . .

DETD . . . deleted for vaccinia genes [C7L-K1L] was assembled in pUC8 cut with SmaI, HindIII and blunt ended with Klenow fragment of **E**. coli polymerase. The left flanking arm consisting of vaccinia HindIII C sequences was obtained by digestion of pSD420 with XbaI (pos. 18,628) followed by blunt ending with Klenow fragment of **E**. coli polymerase and digestion with BglII (pos. 19,706). The right flanking arm consisting of vaccinia HindIII K sequences was obtained. . .

DETD . . . coding sequences, pSD518 was digested with BamHI (pos. 65,381) and HpaI (pos. 67,001) and blunt ended using Klenow fragment of **E**. coli polymerase. This 4.8 kb vector fragment was ligated with a 3.2 kb SmaI cassette containing the **E**. coli Beta-galactosidase gene (Shapira et al., 1983) under the control of the vaccinia 11 kDa promoter (Bertholet et al., 1985;. . .

DETD . . . al., 1989) was inserted into pCE13 by digesting pCE13 with SalI, filling in the sticky ends with Klenow fragment of **E**. coli DNA polymerase and digesting with HindIII. A HindIII--EcoRV fragment containing the H6 promoter sequence was then inserted into pCE13. . .

DETD . . . H6 promoted NDV-F cassette by cloning a HindIII fragment from pCE59 that had been filled in with Klenow fragment of **E**. coli DNA polymerase into the HpaI site of pCE71 to form pCE80. Plasmid pCE80 was completely digested with NdeI and. . .

DETD In NDV-infected cells, the F glycoprotein is anchored in the **membrane** via a hydrophobic transmembrane region near the carboxyl terminus and requires post-translational cleavage of a precursor, F.sub.0, into two disulfide. . .

DETD . . . that immunoreactive proteins were presented on the infected cell surface. To determine that both proteins were presented on the

plasma membrane, monospecific rabbit sera were produced against vaccinia recombinants expressing either the F or HN glycoproteins. Indirect immunofluorescence using these sera. . .

DETD . . . mutagenized expression cassette contained within pRW837 was derived by digestion with HindIII and EcoRI, blunt-ended using the Klenow fragment of **E**. coli DNA polymerase in the presence of 2 mM dNTPs, and inserted into the SmaI site of pSD513 to yield. . .

DETD . . . into pRW843 (containing the measles HA gene). Plasmid pRW843 was first digested with NotI and blunt-ended with Klenow fragment of **E**. coli DNA polymerase in the presence of 2 mM dNTPs. The resulting plasmid, pRW857, therefore contains the measles virus F. . .

DETD Immunoprecipitation reactions were performed as previously described (Taylor et al., 1990) using a guinea-pig anti measles serum (Whittaker **M**. A. Bioproducts, Walkersville, Md.).

DETD . . . mutagenesis was done using MRSYN5 (SEQ ID NO:52) (5'-GCGAGCGAGGCCATGC ATCGTGCGAATGGCCCC-3') and MRSYN6 (SEQ ID NO:53) (5'-GGGGG GACGCGCGGGTCTAGAAGGCCCCGCCTGGCGG-3') and selection on **E**. coli dut.sup.- ung.sup.- strain. CJ236 (International Biotechnologies, Inc., New Haven, Conn.). Mutagenesis was performed according to the protocols of Kunkel. . .

DETD . . . A 1.4 kb fragment containing the I3L promoter/PRV gp50 gene was isolated and blunt-ended using the Klenow fragment of the **E**. coli DNA polymerase in the presence of 2 mM dNTPs.

DETD Vero cells were infected at an **m**.o.i. of 10 pfu per cell with the individual recombinant viruses, with the NYVAC parent virus, or were mock infected. After. . . and were dissociated with RIPA buffer (1% NP-40, 1% Na-deoxycholate, 0.1% SDS, 0.01M methionine, 5 mM EDTA, 5mM 2-mercapto-ethanol, 1 **m**/ml BSA, and 100 u/ml aprotinin). Samples analyzed with sheep anti-gpIII and a monoclonal specific for gp50 were lysed in 1X. . .

DETD Extraneous 3'-noncoding sequence was then eliminated from pGC10. This was accomplished by recircularizing the **E**. coli DNA polymerase I (Klenow fragment) filled-in 4,900 bp SalI-SmaI (partial) fragment of pGC10. The plasmid generated by this manipulation. . .

DETD Extraneous DNA was then eliminated. This was accomplished by cloning the **E**. coli DNA polymerase I (Klenow fragment) filled-in 6,000 bp HindIII-BamHI (partial) fragment of pGBCD1, containing the H6-promoted gB, gC and. . .

DETD Vero cells were infected at an **m**.o.i. of 10 pfu per cell with recombinant vaccinia virus, with the NYVAC parent virus (vP866) or were mock infected. After. . .

DETD . . . inserted individually into three different sites of the virus. The three HBV genes encode the following protein products: (1) HBV **M** protein, (referred to here as small pre S antigen, or spsAg), (2) HBV L protein (referred to here as large. . .

DETD The synthetic S1+S2 region was assembled in five double stranded sections A through **E** as indicated above using synthetic oligonucleotides, MPSYN290 through MPSYN308 (SEQ ID NO:90)-(SEQ ID NO:99), as set out below. Oligonucleotides ranged. . . within a section were kinased before annealing of the section. Sequence of synthetic oligonucleotides used to construct sections A through **E** are given below. Only the coding strand is shown. Relevant restriction sites are noted. Initiation codons for S1 (section A), S2 (section C) and core (section **E**) are underlined. ##STR16## The vaccinia I3L promoter was synthesized using pMP1, a subclone of HindIII I, as template and synthetic. . .

DETD Construction of pRW838 is illustrated below. oligonucleotides A through **E**, which overlap the translation initiation codon of the H6 promoter with the ATG of rabies G, were cloned into pUC9 as pRW737. Oligonucleotides A through **E** contain the H6 promoter, starting at NruI, through the HindIII site of rabies G followed by BglII. Sequences of oligonucleotides A through **E** (SEQ ID NO:109)-(SEQ ID NO. 113) are: ##STR19## The diagram of annealed oligonucleotides A through **E** is as follows: ##STR20##

DETD Oligonucleotides A through **E** were kinased, annealed (95.degree. C. for 5 minutes, then cooled to room temperature), and inserted between the

EVAL SITES OF. . .

DETD . . . stages of assembly of mature rabies virus particles, the glycoprotein component is transported from the golgi apparatus to the plasma **membrane** where it accumulates with the carboxy terminus extending into the cytoplasm and the bulk of the protein on the external surface of the cell **membrane**. In order to confirm that the rabies glycoprotein expressed in ALVAC-RG was correctly presented, immunofluorescence was performed on primary CEF. . .

DETD The initial inoculation was performed at an **m**.o.i. of 0.1 pfu per cell using three 60 mm dishes of each cell line containing 2.times.10.sup.6 cells per dish. One. . .

DETD . . . parental canarypox virus, (b) ALVAC-RG, the recombinant expressing the rabies G glycoprotein or (c) vCP37, a canarypox recombinant expressing the **envelope** glycoprotein of feline leukemia virus. Inoculations were performed under ketamine anaesthesia. Each animal received at the same time: (1) 20. . .

DETD (**e**) Primary CEF cells.

DETD . . . electrophoresis the viral DNA band was visualized by staining with ethidium bromide. The DNA was then transferred to a nitrocellulose **membrane** and probed with a radiolabelled probe prepared from purified ALVAC genomic DNA.

DETD . . . seed 23 3.34

Vaccine Batch H
23 4.52

Vaccine Batch I
23 3.33

Vaccine Batch K
15 3.64

Vaccine Batch L
15 4.03

Vaccine Batch **M**
15 3.32

Vaccine Batch N
15 3.39

Vaccine Batch J
23 3.42

.sup.a Expressed as mouse LD.sub.50

.sup.b Expressed as log.sub.10 TCID.sub.50

DETD . . . 2.1 2.2 .sup. N.T..sup.g

55	vCP37.sup.d	NT	<1.2	<1.2	1.7	2.2	2.1	N.T.
37	ALVAC-RG.sup.**e**	2.2	<1.2	<1.2	3.2	3.5	3.5	3.2
53	ALVAC-RG.sup.**e**	2.2	<1.2	<1.2	3.6	3.6	3.6	3.4
38	ALVAC-RG.sup.f	2.7	<1.7	<1.7	3.2	3.8	3.6	N.T.
54	ALVAC-RG.sup.f	3.2	<1.7	<1.5	3.6	4.2	4.0	3.6
57	None	NT. . . 28 after primary vaccination						

.sup.c Animals received 5.0 log.sub.10 TCID.sub.50 of ALVAC

.sup.d Animals received 5.0 log.sub.10 TCID.sub.50 of vCP37

.sup.**e** Animals received 5.0 log.sub.10 TCID.sub.50 of ALVACRG

.sup.f Animals received 7.0 log.sub.10 TCID.sub.50 of ALVACRG

.sup.g Not tested.

DETD TABLE 15

Inoculation of chimpanzees with ALVAC-RG

Weeks post-Inoculation

	Animal 431 I.**M**.	Animal 457 S.C.
0	.sup. <8.sup.a	<8
1	<8	<8

2	8	32
4	16	32
8	16	32
12.sup.b /0	16	8
13/1	128	128
15/3	256. . .	

DETD Construction of NYVAC Recombinants Expressing **Flavivirus** Proteins

DETD This example describes the construction of NYVAC donor plasmids containing genes from Japanese **encephalitis virus** (JEV), **yellow fever virus** (YF) and **Dengue** type 1, the isolation of the corresponding NYVAC **Flavivirus** recombinants and the ability of vaccinia recombinants expressing portions of the genomes of JEV or YF to protect mice against. . .

DETD . . . and AccI fragment of JEV2 (Mason et al., 1991) containing the plasmid origin and JEV cDNA encoding the carboxy-terminal 40% **prM** and amino-terminal two thirds of **E** (nucleotides 602 to 2124), generating plasmid JEV20 containing JE sequences from the ATG of C through the SacI site (nucleotide 2124) found in the last third of **E**.

DETD . . . 1991) in which TTTTTGT nucleotides 1304 to 1310 were changed to TCTTTGT), containing JE sequences from the last third of **E** through the first two amino acids of NS2B (nucleotides 2124 to 4126), the plasmid origin and vaccinia sequences, was ligated. . .

DETD . . . end] generated plasmid JEV25 which contains JE cDNA extending from the SacI site (nucleotide 2124) in the last third of **E** through the carboxy-terminus of **E**. The SacI-EagI fragment from JEV25 was ligated to the SacI-EagI fragment of JEV8 (containing JE cDNA encoding 15 aa C, **prM** and amino-terminal two thirds of **E** nucleotides 337 to 2124, the plasmid origin and vaccinia sequences) yielding plasmid JEV26. A unique SmaI site preceding the ATG. . .

DETD . . . fragment from JEV7 (Mason et al., 1991) yielded JEV29 (containing a SmaI site followed by JE cDNA encoding 30 aa **E**, NS1, NS2A nucleotides 2293 to 4126) and JEV30 (containing a SmaI site followed by JE cDNA encoding 30 aa **E**, NS1, NS2A, NS2B nucleotides 2293 to 4512).

DETD HeLa cell monolayers were prepared in 35 mm diameter dishes and infected with vaccinia viruses (**m**.o.i. of 2 pfu per cell) or JEV (**m**.o.i. of 5 pfu per cell) before radiolabeling. Cells were pulse labeled with medium containing .sup.35 S-Met and chased for 6. . .

DETD Recombinant vP825 encoded the capsid protein, structural protein precursor **prM**, the structural glycoprotein **E**, the nonstructural glycoprotein NS1, and the nonstructural protein NS2A (McAda et al., 1987). Recombinant vP829 encoded the putative 15 aa **signal sequence** preceding the amino-terminus of **prM**, as well as **prM**, and **E** (McAda et al., 1987). Recombinant vP857 contained a cDNA encoding the 30 aa hydrophobic carboxy-terminus of **E**, followed by NS1 and NS2A. Recombinant vP864 contained a cDNA encoding the same proteins as vP857 with the addition of NS2B. In recombinants vP825 and vP829 a potential vaccinia virus early transcription termination signal in **E** (TTTTTGT; nucleotides 1304-1310) was modified to TCTTTGT without altering the aa sequence. This change was made in an attempt to increase the level of expression of **E** since this sequence has been shown to increase transcription termination in in vitro transcription assays (Yuen et al., 1987).

DETD **E** and **prM** Are Correctly Processed When Expressed By Recombinant Vaccinia Viruses

DETD Pulse-chase experiments demonstrate that proteins identical in size to **E** were synthesized in cells infected with all recombinant vaccinia viruses containing the **E** gene (Table 16). In the case of cells infected with JEV, vP555 and vP829, an **E** protein that migrated slower in SDS-PAGE was also detected in the culture fluid harvested from the infected cells (Table 16). This extracellular form of **E** produced by JEV- and vP555-infected cells contained mature N-linked glycans (Mason, 1989; Mason et al., 1991), as confirmed for the extracellular forms of **E** produced by vP829-infected cells. Interestingly, vP825, which contained the C coding region in addition to **prM** and **E** specified the synthesis of **E** in a form that is not released into the extracellular

fluid (Table 16). Immunoprecipitations prepared from radiolabeled recombinant vaccinia-infected cells using a MAb specific for **M** (and **prM**) revealed that **prM** was synthesized in cells infected with vP555, vPS25, and vP829, and **M** was detected in the culture fluid of cells infected with vP555 or vP829 (Table 16).

DETD . . . (data not shown). This result indicated that vP829 infected cells produce extracellular particles similar to the empty viral envelopes containing **E** and **M** observed in the culture fluids harvested from vP555 infected cells (Table 16 and Mason et al., 1991).

DETD Pre-challenge sera pooled from selected animals in each group were tested for their ability to immunoprecipitate radiolabeled **E** and NS1. The results of these studies (Table 16) demonstrated that: (1) the magnitude of immune response induced to **E** was vP829>vP555>vP825, (2) all viruses encoding NS1 and NS2A induced antibodies to NS1, and (3) all immune responses were increased. . . sera collected from these animals (Table 17) confirmed the results of the immunoprecipitation analyses, showing that the immune response to **E** as demonstrated by RIP correlated well with these other serological tests (Table 17).

DETD TABLE 16

Characterization of proteins expressed by vaccinia recombinants expressing JEV proteins and their immune responses

	vP555	vP829	vP825	vP857	vP864
Proteins expressed.sup.a					
Intracellular	**prM, E**	**prM, E**	**prM, E** NS1	NS1	NS1
secreted	NS1 **M, E,** NS1		NS1		
		M, E	none	NS1	NS1
Particle formation.sup.b	+	+	-	-	-
Immune response.sup.c					
single	**E**	**E**	NS1	NS1	NS1
double	**E,** NS1	**E**	**E,** NS1 NS1	NS1	NS1

.sup.a Radiolabelled cell lysates and culture fluids from vaccinia virus JEV recombinant infected cells were harvested and JEVspecific proteins immunoprecipitated using mAbs to **E, M** and NS1 proteins.

.sup.b Formation of extracellular particles with HA activity as described in the text.

.sup.c JEV proteins were. . .

DETD . . . isolated and ligated to a SacI (JEV nucleotide 2124) to EagI fragment of JEV25 (containing the remaining two thirds of **E**, translation stop and T5NT) generating JEV36. JEV36 was transfected into vP866 (NYVAC) infected cells to generate the vaccinia recombinant vP923. . .

DETD Plasmid YFO containing YF cDNA encoding the carboxy-terminal 80% **prM, E** and amino-terminal 80% NS1 (nucleotides 537-3266) was derived by cloning an AvaI to NsiI fragment of YF cDNA (nucleotides 537-1659). . . and EpnI digested IBI25 (International Biotechnologies, Inc., New Haven, Conn.). Plasmid YF1 containing YF cDNA encoding C and amino-terminal 20% **prM** (nucleotides 119-536) was derived by cloning a RsaI to AvaI fragment of YF cDNA (nucleotides 166-536) and annealed oligonucleotides SP46. . . and YF nucleotides 119-165) into AvaI and HindIII digested IBI25. Plasmid YF3 containing YF cDNA encoding the carboxy-terminal 60% of **E** and amino-terminal 25% of NS1 was generated by cloning an ApaI to BamHI fragment of YF cDNA (nucleotides 1604-2725) into. . . cDNA (nucleotides 4339-4940) into SacI and XbaI digested IBI25. Plasmid YF13 containing YF cDNA encoding the carboxy-terminal 25% of C, **prM** and amino-terminal 40% of **E** was derived by cloning a BalI to ApaI fragment of YF cDNA (nucleotides 384-1603) into ApaI and SmaI digested IBI25.

DETD . . . in YF1 (TTTTTCT nucleotides 263-269 and TTTTTGT nucleotides

2057-2757; to (SEQ ID NO:122) nucleotides creating plasmid YF3B; (2) in the **E** gene in YF3 (nucleotides 1886-1893 TTTTTTGT to TTCTTTGT 189 aa from the carboxy-terminus and nucleotides 2429-2435 TTTTTGT to TTCTTGT 8. . . YF3C (nucleotides 1965-2725) was exchanged for the corresponding fragment of YF3B generating YF4 containing YF cDNA encoding the carboxy-terminal 60% **E** and amino-terminal 25% NS1 (nucleotides 1604-2725) with both mutagenized transcription termination signals. An ApaI to BamHI fragment from YF4 (nucleotides 1604-2725) was substituted for the equivalent region in YF0 creating plasmid YF6 containing YF cDNA encoding the carboxy-terminal 80% **prM**, **E** and amino-terminal 80% NS1 (nucleotides 537-3266) with both mutagenized transcription termination signals. Plasmid YF6 was digested with EcoRV within the. . .

DETD . . . described above was used (1) to insert XhoI and ClaI sites preceding the ATG 17 aa from the carboxy-terminus of **E** (nucleotides 2402-2404) in plasmid YF3C creating YF5, (2) to insert XhoI and ClaI sites preceding the ATG 19 aa from the carboxy-terminus of **prM** (nucleotides 917-919) in plasmid YF13 creating YF14, (3) to insert an XhoI site preceding the ATG 23 aa from the carboxy-terminus of **E** (nucleotides 2384-2386) in plasmid YF3C creating plasmid YF25, (4) and to insert an XhoI site and ATG (nucleotide 419) in. . .

DETD . . . YF5 (nucleotides 1604-2725) was exchanged for the corresponding region of YF1 creating YF7 containing YF cDNA encoding the carboxy-terminal 80% **prM**, **E** and amino-terminal 80% NS1 (nucleotides 537-3266) with XhoI and ClaI sites at 2402 (17 aa from the carboxy-terminus of **E**) and a mutagenized transcription termination signal at 2429-2435 (8 aa from the carboxy-terminus of **E**). The ApaI to BamHI fragment from YF25 (nucleotides 1604-2725) was exchanged for the corresponding region of YF0 generating YF26 containing YF cDNA encoding the carboxy-terminal 80% **prM**, **E** and amino-terminal 80% NS1 (nucleotides 537-3266) with an XhoI site at nucleotide 2384 (23 aa from the carboxy-terminus of **E**) and mutagenized transcription termination signal at 2428-2435 (8 aa from the carboxy-terminus of **E**).

DETD . . . YF14 (nucleotides 537-1603) was substituted for the corresponding region in YF6 generating YF15 containing YF cDNA encoding the carboxy-terminal 80% **prM**, **E** and amino-terminal 80% NS1 (nucleotides 537-3266) with XhoI and ClaI sites at nucleotide 917 (19 aa from the carboxy-terminus of **prM**) and two mutagenized transcription termination signals. YF6 was digested within IBI25 with EcoRV and within YF at nucleotide 537 with. . .

DETD . . . from YF12 encoding carboxy-terminal 20% NS1, NS2A and NS2B (nucleotides 3267-4569), XhoI to KpnI fragment from YF15 encoding 19 aa **prM**, **E** and amino-terminal 80% NS1 (nucleotides 917-3266) and XhoI-SmaI digested pHES4 were ligated generating YF23. An XhoI to BamHI fragment from YF26 encoding 23 aa **E**, amino-terminal 25% NS1 (nucleotides 2384-2725) was ligated to an XhoI to BamHI fragment from YF23 (containing the carboxy-terminal 75% NS1,. . .

DETD XhoI-SmaI digested pHES4 was ligated to a purified XhoI to KpnI fragment from YF7 encoding 17 aa **E** and amino-terminal 80% NS1 (nucleotides 2402-3266) plus a KpnI to SmaI fragment from YF10 encoding the carboxy-terminal 20% NS1 and NS2A (nucleotides 3267-4180) creating YF18. An XhoI to BamHI fragment from YF2 encoding C, **prM**, **E** and amino-terminal 25% NS1 (nucleotides 119-2725) was ligated to a XhoI to BamHI fragment of YF18 (containing the carboxy-terminal 75%. . . the origin of replication and vaccinia sequences) generating YF20. A XhoI to BamHI fragment from YF46 encoding 21 aa C, **prM**, **E** and amino-terminal 25% NS1 (nucleotides 419-2725) was ligated to the XhoI to BamHI fragment from YF18 generating YF47. Oligonucleotide SP46. . .

DETD Recombinant vP725 encoded the putative 17-aa **signal sequence** preceding the N terminus of the nonstructural protein NS1 and the nonstructural proteins NS1 and NS2A (Rice et al., 1985). Recombinant vP729 encoded the putative 19-aa **signal sequence** preceding the N terminus of **E**, **E**, NS1, NS2A and NS2B (Rice et al., 1985). Recombinant vP764 encoded C, **prM**, **E**, NS1, NS2A and NS2B (Rice et al., 1985). Recombinant vP766 encoded C, **prM**, **E**, NS1 and NS2A (Rice et al., 1985). Recombinant vP869 encoded the putative 21-aa **signal**

sequence preceding the N terminus of the **prM** structural protein precursor as well as **prM**, **E**, NS1 and NS2A (Rice et al., 1985).

DETD A XhoI to SmaI fragment from YF47 (nucleotides 419-4180) containing YF cDNA encoding 21 amino acids C, **prM**, **E**, NS1, NS2A (with nucleotide 2962 missing in NS1) was ligated to XhoI-SmaI digested SPHA-H6 (HA region donor plasmid) generating YF48.. . . (nucleotide 3262) and a 6700 bp fragment isolated (containing the plasmid origin of replication, vaccinia sequences, 21 amino acids C, **prM**, **E**, amino-terminal 3.5% NS1, carboxy-terminal 23% NS1, NS2A) and ligated to a SacI-Asp718 fragment from YF18 (containing the remainder of NS1. . . in YF51 were removed using oligonucleotide-directed double-strand break mutagenesis (Mandecki, 1986) creating plasmid YF50 encoding YF 21 amino acids C, **prM**, **E**, NS1, NS2A in the HA locus donor plasmid. Donor plasmid YF50 was transfected into vP866 (NYVAC) infected cells to generate. . .

DETD . . . double-strand break mutagenesis creating YF49. Oligonucleotide-directed mutagenesis (Kunkel, 1985) was used to insert a SmaI site at the carboxy-terminus of **E** (nucleotide 2452) in YF4 creating YF16. An ApaI-SmaI fragment of YF49 (containing the plasmid origin of replication, vaccinia sequences and YF cDNA encoding 21 amino acid C, **prM**, and amino-terminal 43% **E**) was ligated to an ApaI-SmaI fragment from YF16 (nucleotides 1604-2452 containing the carboxy-terminal 57% **E**) generating YF53 containing 21 amino acids of C, **prM**, **E** in the HA locus. Donor plasmid YF53 was transfected into vP913 (NYVAC-MV) infected cells to generate the vaccinia recombinant vP997.

DETD . . . digested pUC8. An EcoRI-HindIII fragment from DEN1 (nucleotides 2559-3745) and SacI-EcoRI fragment of DEN cDNA encoding the carboxy-terminal 36% of **E** and amino-terminal 16% NS1 (nucleotides 1447-2559, Mason et al., 1987b) were ligated to HindIII -SacI digested IBI24 (International Biotechnologies, Inc., New Haven, Conn.) generating DEN3 encoding the carboxy-terminal 64% **E** through amino-terminal 45% NS2A with a base missing in NS1 (nucleotide 2467).

DETD . . . an AvaI-SacI fragment of DEN cDNA (nucleotides 424-1447 Mason et al., 1987) generating DEN4 encoding the carboxy-terminal 11 aa C, **prM** and amino-terminal 36% **E**.

DETD Plasmid DEN6 containing DEN cDNA encoding the carboxy-terminal 64% **E** and amino-terminal 18% NS1 (nucleotides 1447-2579 with nucleotide 2467 present Mason et al., 1987b) was derived by cloning a SacI-XhoI. . . Biotechnologies, Inc., New Haven, Conn.). Plasmid DEN15 containing DEN cDNA encoding 51 bases of the DEN 5' untranslated region, C, **prM** and amino-terminal 36% **E** was derived by cloning a HindIII-SacI fragment of DEN cDNA (nucleotides 20-1447, Mason et al., 1987b) into HindIII-SacI digested IBI25.. . .

DETD . . . change the following potential vaccinia virus early transcription termination signals (Yuen et al., 1987). The two T5NT seqeunces in the **prM** gene in DEN4 were mutagenized (1) 29 aa from the carboxy-terminus (nucleotides 822-828 TTTTTCT to TATTTCT) and (2) 13 aa. . .

DETD . . . 4102) in plasmid DEN23 creating DEN24, (2) to insert a SmaI site and ATG 15 aa from the carboxy-terminus of **E** in DEN7 (nucleotide 2348) creating DEN10, (3) to insert an EagI and HindIII site at the carboxy-terminus of NS2B (nucleotide. . .

DETD . . . DEN7 (nucleotides 1447-2579) was substituted for the corresponding region in DEN3 generating DEN19 containing DEN cDNA encoding the carboxy-terminal 64% **E** and amino-terminal 45% NS2A (nucleotides 1447-3745) with nucleotide 2467 present and the modified transcription termination signal (nucleotides 2448-2454). A XhoI-XbaI. . .

DETD A HindIII-PstI fragment of DEN16 (nucleotides 20-494) was ligated to a HindIII-PstI fragment from DEN47 (encoding the carboxy-terminal 83% **prM** and amino-terminal 36% of **E** nucleotides 494-1447 and plasmid origin of replication) generating DEN17 encoding C, **prM** and amino-terminal 36% **E** with part of the H6 promoter and EcoRV site preceding the amino-terminus of C. A HindIII-BglII fragment from DEN17 encoding the carboxy-terminal 13 aa C, **prM** and amino-terminal 36% **E** (nucleotides 370-1447) was ligated to annealed oligonucleotides SP111

and SF11Z (containing a disabled HindIII sticky end, EcoRV site to -1. . . a BglII sticky end) creating DEN33 encoding the EcoRV site to -1 of the H6 promoter, carboxy-terminal 20 aa C, **prM** and amino-terminal 36% **E**.

DETD . . . digested pTP15 (Mason et al., 1991) was ligated to a SmaI-SacI fragment from DEN4 encoding the carboxy-terminal 11 aa C, **prM** and amino-terminal 36% **E** (nucleotides 377-1447) and SacI-EagI fragment from DEN3 encoding the carboxy-terminal 64% **E**, NS1 and amino-terminal 45% NS2A generating DENL. The SacI-XhoI fragment from DEN7 encoding the carboxy-terminal 64% **E** and amino-terminal 18% NS1 (nucleotides 1447-2579) was ligated to a BstEII-SacI fragment from DEN47 (encoding the carboxy-terminal 55% **prM** and amino-terminal 36% **E** nucleotides 631-1447) and a BstEII-XhoI fragment from DENL (containing the carboxy-terminal 11 aa C, amino-terminal 45% **prM**, carboxy-terminal 82% NS1, carboxy-terminal 45% NS2A, origin of replication and vaccinia sequences) generating DEN8. A unique SmaI site (located between. . .

DETD . . . an EcoRV-SacI fragment of DEN8VC (containing vaccinia sequences, H6 promoter from -21 to -124, origin of replication and amino-terminal 64% **E**, NS1, amino-terminal 45% NS2A nucleotides 1447-3745) generating DEN18. A XhoI-EagI fragment from DEN25 (nucleotides 2579-4102) was ligated to an XhoI-EagI fragment of DEN18 (containing the origin of replication, vaccinia sequences and DEN C, **prM**, **E** and amino-terminal 18% NS1 nucleotides 68-2579) generating DEN26. An EcoRV-SacI fragment from DEN8VC (positions -21 to -1 H6 promoter DEN. . . to an EcoRV-SacI fragment of DEN26 (containing the origin of replication, vaccinia sequences and DEN region encoding the carboxy-terminal 64% **E**, NS1 and NS2A with a base missing in NS1 at nucleotide 2894) generating DEN32. DEN32 was transfected into vP410 infected. . .

DETD . . . DEN10 (nucleotides 1447-2579) was substituted for the corresponding region in DEN3 generating DEN11 containing DEN cDNA encoding the carboxy-terminal 64% **E**, NS1 and amino-terminal 45% NS2A with a SmaI site and ATG 15 aa from the carboxy-terminus of **E**. A SmaI-EagI fragment from DEN11 (encoding the carboxy-terminal 15 aa **E**, NS1 and amino-terminal 45% NS2A nucleotides 2348-3745) was ligated to SmaI-EagI digested pTP15 generating DEN12.

DETD An EcoRV-XhoI fragment from DEN8VC (positions -21 to -1 H6 promoter DEN nucleotides 377-2579 encoding the carboxy-terminal 11 aa C, **prM E**, amino-terminal 18% NS1) was ligated to an EcoRV-XhoI fragment from DEN31 (containing the origin of replication, vaccinia sequences and DEN. . . An EcoRV-SacI fragment from DEN33 (positions -21 to -1 H6 promoter DEN nucleotides 350-1447 encoding the carboxy-terminal 20 aa C, **prM** and amino-terminal 36% **E**) and a SacI-XhoI fragment from DEN32 (encoding the carboxy-terminal 64% **E** and amino-terminal 18% NS1 nucleotides 1447-2579) were ligated to the EcoRV-SacI fragment from DEN31 described above generating DEN34. DEN34 was. . .

DETD . . . the left terminus of vaccinia and by introducing a deletion near the right terminus. All deletions were accomplished using the **E**. coli guanine phosphoribosyl transferase gene and mycophenolic acid in a transient selection system.

DETD For use as a selectable marker, the **E**. coli gene encoding guanine phosphoribosyl transferase (Ecogpt) (Pratt et al., 1983) was placed under the control of a poxvirus promoter.. . .

DETD . . . subunit of ribonucleotide reductase (Slabaugh et al., 1988). Also included in this deletion is ORF F2L, which shows homology to **E**. coli dUTPase, another enzyme involved in nucleotide metabolism (Goebel et al., 1990a,b). F2L also shows homology to retroviral protease (Slabaugh. . .

DETD . . . sequences, the predicted translation product of Copenhagen ORF B16 is truncated at the amino terminus and does not contain a **signal sequence**. B19R encodes a vaccinia surface protein (S antigen) expressed at early times post infection (Ueda et al., 1990). Both B16R. . .

DETD . . . immunological assays was comprised of RPMI 1640 medium supplemented with 10% FBS, 4 mM L-glutamine, 20 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonate), 5.times.10.sup.-5 **M**

2 mercaptoethanol, 100 IU penicillin per ml, and 100 .mu.g/ml streptomycin. Stim Medium was comprised of Eagle's Minimum Essential Medium supplemented with 10% FBS, 4 mM L-glutamine, 10.sup.-4 **M** 2-mercaptoethanol, 100 IU penicillin per ml, and 100 .mu.g streptomycin per ml.

DETD ALVAC and NYVAC Recombinants Containing the V3 Loop and Epitope 88 of the HIV-1 (IIIB) **Envelope**

DETD . . . isolated by phenol extraction (2.times.) and ether extraction (1.times.). The isolated fragment was blunt-ended using the Klenow fragment of the **E**. coli DNA polymerase in the presence of 2 mM dNTPs. The fragment was ligated to pSD550, a derivative of pSD548. . .

DETD ALVAC- and NYVAC-Based Recombinants Expressing the HIV-1 (IIIB) **Envelope** Glycoproteins

DETD . . . pBSHIV3BEAII was digested with NruI and XbalI. The derived 2.7 kb fragment was blunt-ended with the Klenow fragment of the **E**. coli DNA polymerase in the presence of 2 mM dNTPs. This fragment contains the entire HIV-1 env gene juxtaposed 3'. . .

DETD . . . followed by a partial KpnI digestion. The 1.6 kb fragment was blunt-ended by treatment with the Klenow fragment of the **E**. coli DNA polymerase in the presence of 2 mM dNTPs. This fragment was inserted into pSD54IVC digested with SmaI to. . .

DETD . . . Vero cells monolayers were either mock infected, infected with the parental virus vP866, or infected with recombinant virus at an **m**.o.i. of 10 PFU/cell. Following a 1 hr adsorption period, the inoculum was aspirated and the cells were overlayed with 2. . .

DETD . . . using sera pooled from HIV-1 seropositive individuals showed specific precipitation of the gp120 and gp41 mature forms of the gp160 **envelope** glycoprotein from vP911 infected cell lysates. No such specific gene products were detected in the parentally (NYVAC; vP866) infected cell. . .

DETD . . . for 1 hour in tissue culture medium containing 2% FBS at 37.degree. C. with the appropriate vaccinia virus at a **m**.o.i. of 25 pfu per cell. Following infection, the stimulator cells were washed several times in Stim Medium and diluted to. . .

DETD . . . cells were infected overnight by incubation at 1.times.10.sup.7 cells per ml in tissue culture medium containing 2% FBS at a **m**.o.i. of 25 pfu per cell for 1 hour at 37.degree. C. Following incubation, the cells were diluted to between 1-2.times.10.sup.6. . .

DETD . . . 1.8 2.2 1.2

vP911	-4.0	4.6 *	1.4
	.+-. 2.5	2.0	5.1
vP921	-3.4	10.7 *	15.5 *
	.+-. 0.9	1.5	2.8

E:T = 100:1

* P < 0.05 vs appropriate controls, Student's ttest

DETD . . . plasmid vector, pIBI25 (International Biotechnologies, Inc., New Haven, Conn.), generating plasmid pIBI25env. Recombinant plasmid pIBI25env was used to transform competent **E**. coli CJ236 (dut- ung-) cells. Single-stranded DNA was isolated from phage derived by infection of the transformed **E**. coli CJ236 cells with the helper phage, MG408. This single-stranded template was used in vitro mutagenesis reactions (Kunkel et al.,. . .

DETD . . . 2.5 kb (envIS+) and 2.4 kb (envIS-), respectively, were isolated and blunt-ended by reaction with the Klenow fragment of the **E**. coli DNA polymerase in the presence of 2 mMdNTPs. These fragments were ligated with the 3.5 kb fragment derived by digestion of pSIVenvVV with NruI and PstI with a subsequent blunting step with the Klenow fragment of the **E**. coli DNA polymerase in the presence of 2 mM dNTPs. The plasmid pSIVenvVV contains the SIV env gene expression cassette. .

.

DETD . . . seropositive individuals were performed as described in Materials and Methods. All six recombinants directed the synthesis of the HIV-1 gp161 **envelope** precursor. The efficiency of processing of gp160 to gp120 and gp41, however, varied between cell types and was also affected. . .

DETD . . . to yield pBSH6HIV2ENV. The 2.7 kb HindIII/XbaI insert from pBSH6HIV2ENV was isolated and blunt-ended with the Klenow fragment of the **E**. coli DNA polymerase in the presence of 2 mM DNTP. The blunt-ended fragment was inserted into a SmaI digested pSD5HIVC. . .

DETD . . . gp160. Vero cell monolayers were either mock infected, infected with the parental virus vP866, or infected with vP920 at an **m**.o.i. of 10 PFU/cell. Following an hour adsorption period, the inoculum was aspirated and the cells were overlayed with 2 mls. . .

DETD Human sera from HIV-2 seropositive individuals specifically precipitated the HIV-2 gp160 **envelope** glycoprotein from vP920 infected cells. Furthermore, the authenticity of the expressed HIV-2 env gene product was confirmed, since the gp160. . .

DETD . . . coding sequence juxtaposed 3' to the vaccinia virus H6 promoter. This fragment was blunted with the Klenow fragment of the **E**. coli DNA polymerase in the presence of 2 mM dNTPs. The blunt-ended fragment was ligated to SmaI digested pSDSHIVC to. . .

DETD . . . digestion with HindIII liberated a 2.7 kb HindIII/EcoRI fragment. This fragment was blunt-ended by treatment with Klenow fragment of the **E**. coli DNA polymerase in the presence of 2 mM dNTPs. The fragment was ligated into pSD55OVC digested with SmaI. The. . .

DETD The SIV gp140 env gene product is a typical glycoprotein associated with the plasma **membrane** of infected cells. It is expressed as a polyprotein of 140 kDa that is proteolytically cleaved to an extracellular species. . .

DETD . . . and gag) in Vero cells infected with the NYVAC/HIV recombinants was analyzed by immunoprecipitation. Vero cells were infected at an **m**.o.i. of 10 with the individual recombinant viruses, with the NYVAC parent virus, or were mock infected. After a 1 hour. . .

DETD The plasmid pF7D3 was linearized with XhoI and blunt-ended with the Klenow fragment of the **E**. coli DNA polymerase in the presence of 2 mM dNTPs. This linearized plasmid was ligated with annealed oligonucleotides F7MCSB (SEQ. . .

DETD . . . the H6 promoter) and PstI. The 3.5 kb resultant fragment was isolated and blunt-ended using the Klenow fragment of the **E**. coli DNA polymerase in the presence of 2 mM dNTPs. This blunt-ended fragment was ligated to a 1700 bp EcoRV/HpaI. . .

DETD . . . HA molecule is synthesized and glycosylated as a precursor molecule at the rough endoplasmic reticulum. During passage to the plasma **membrane** it undergoes extensive post-translational modification culminating in proteolytic cleavage into the disulphide linked HA1 and HA2 subunits and insertion into the host cell **membrane** where it is subsequently incorporated into mature viral envelopes. To determine whether the HA molecules produced in cells infected with. . .

DETD . . . 3'end-EcoRV fragment (D). Plasmid pVHAH6g13 was digested with BglII and KpnI to isolate the 1330 bp BglII-H6-EHV-1 gC 5'-KpnI fragment (**E**).

DETD Fragments C, D and **E** were finally ligated together into vector pSD541VC digested with BglII and XhoI to produce plasmid pJCA042. Plasmid pJCA042 is the. . .

DETD . . . region-BamHI fragment (L). Plasmid pVHAH6g13 was digested with BglII and XhoI to isolate the 440 bp BglII-H6-EHV-1 gC 5'portion-XhoI fragment (**M**). Fragments K, L and **M** were then ligated together to produce plasmid pJCA040.

DETD . . . authentic BHV1 gIV glycoprotein. Vero cell monolayers were either mock infected, infected with NYVAC or infected with vP1051 at an **m**.o.i. of 10 PFU/cell. Following an hour adsorption period, the inoculum was aspirated and the cells were overlayed with 2 mls. . .

DETD . . . gene was then cloned into pIBR8. This was accomplished by cloning the 2,285 bp StuI fragment of pIBRS6 into the **E**. coli DNA polymerase I (Klenow fragment) filled-in 4,300 bp StuI-BglII (partial) fragment of pIBR8. The plasmid generated by this manipulation. . .

DETD The H6-promoted BHV1 gI gene was then moved to a vaccinia virus donor plasmid. This was accomplished by cloning the **E**. coli DNA polymerase I (Klenow fragment) filled-in 2,900 bp BglII-NcoI (partial) fragment of pIBR20 into the SmaI site of pSD542.. . .

DETD . . . gI and gIV glycoproteins. Vero cell monolayers were either mock

infected, infected with NYVAC or infected with vP1074 at an **m**.o.i. of 10 PFU/cell. Following an hour adsorption period, the inoculum was aspirated and the cells were overlayed with 2 mls. . .

DETD . . . authentic BHV1 gIII glycoprotein. Vero cell monolayers were either mock infected, infected with NYVAC or infected with vP1073 at an **m**.o.i. of 10 PFU/cell. Following an hour adsorption period, the inoculum was aspirated and the cells were overlayed with 2 mls. . .

DETD . . . gIII and gIV glycoproteins. Vero cell monolayers were either mock infected, infected with NYVAC or infected with vP1083 at an **m**.o.i. of 10 PFU/cell. Following an hour adsorption period, the inoculum was aspirated and the cells were overlayed with 2 mls. . .

DETD . . . gI and gIII glycoproteins. Vero cell monolayers were either mock infected, infected with NYVAC or infected with vP1087 at an **m**.o.i. of 10 PFU/cell. Following an hour adsorption period, the inoculum was aspirated and the cells were overlayed with 2 mls. . .

DETD . . . gIII and gIV glycoproteins. Vero cell monolayers were either mock infected, infected with NYVAC or infected with vP1079 at an **m**.o.i. of 10 PFU/cell. Following an hour adsorption period, the inoculum was aspirated and the cells were overlayed with 2 mls. . .

DETD . . . the 1,370 bp EcoRI-BamHI fragment of pSP65-gE1 (obtained from Eurogentec, Liege, Belgium; Renard et al., European Patent Application No:86870095) with **E**. coli DNA polymerase I (Klenow fragment), ligating XhoI linkers onto the ends and cloning the resulting fragment into the XhoI. . .

DETD . . . gE1 and gE2 glycoproteins. Vero cell monolayers were either mock infected, infected with NYVAC or infected with vP972 at an **m**.o.i. of 10 PFU/cell. Following an hour adsorption period, the inoculum was aspirated and the cells were overlayed with 2 mls. . .

DETD . . . cloned into pIBI25. This was accomplished by blunt-ending the 1,370 bp EcoRI-BamHI fragment of pSP65-gE1, containing the gE1 "gene", with **E**. coli DNA polymerase I (Klenow fragment), ligating XhoI linkers onto the ends and cloning the resulting fragment into the XhoI. . .

DETD . . . cloned into pIBI25. This was accomplished by blunt-ending the 1,370 bp EcoRI-BamHI fragment of pSP65-gE1, containing the gE1 "gene", with **E**. coli DNA polymerase I (Klenow fragment), ligating XhoI linkers onto the ends and cloning the resulting fragment into the XhoI. . .

DETD . . . pig polyclonal serum followed by fluorescein isothiocyanate goat anti-guinea pig. Cells infected with vP1001 showed gB expressed on the plasma **membrane**. Weak internal expression was detected within cells infected with vCP139.

DETD . . . gene was excised from pED3 with NruI and XhoI and the purified fragment was cloned into pVQH6CP3L (plasmid described in **Flavivirus** section) cut with NruI and XhoI. The resulting plasmid, pC3-VP2, contains the H6 promoted VP2 gene flanked by the C3. . .

DETD . . . with EcoRI, which recognizes a unique EcoRI site within the canarypox sequences, and blunt-ended using the Klenow fragment of the **E**. coli DNA polymerase. The resultant plasmid was designated as pCPCV1. This plasmid contains the vaccinia virus H6 promoter followed by. . .

DETD An M13 clone containing the hemagglutinin (HA) gene from equine influenza virus (A2/Suffolk/89) was provided by Dr. **M**. Binns (Animal Health Trust, P.O. Box 5, Newmarket, Suffolk, CB8 7DW, United Kingdom). This clone contains a full-length 1.7 kb. . .

DETD . . . recombination tests with vP425 as the rescuing virus to construct a recombinant vaccinia virus (vP453) which expresses the entire FeLV **envelope** glycoprotein.

DETD . . . tests with vP410 as the rescuing virus to generate vP456. This vaccinia virus recombinant was generated to express the entire **envelope** glycoprotein lacking the putative immunosuppressive region.

DETD . . . of the H6 promote sequence. The PstI site is located 420 bp downstream from the translation termination signal for the **envelope** glycoprotein open reading frame.

DETD . . . of the H6 promoter sequence. The HpaI site is located 180 bp downstream from the translation termination signal for the **envelope** glycoprotein open reading frame. These isolated fragments were blunt-ended. These 2.2 kbp H6/FeLV env sequences were inserted into the

nonessential. . .

DETD . . . with EcoRI, which recognizes a unique EcoRI site within the canarypox sequences, and blunt-ended using the Klenow fragment of the **E**. coli DNA polymerase. The resultant plasmid was designated as pCPCV1. This plasmid contains the vaccinia virus H6 promoter followed by. . .

DETD The putative immunosuppressive region is situated within the p15E transmembrane region of the FeLV **envelope** glycoprotein (Cianciolo et al., 1986; Mathes et al., 1978). This region was deleted in the following manner. The FeLV-A env. . .

DETD . . . into the SmaI site of pSD553. This insertion was performed following blunt-ending the fragment with the Klenow fragment of the **E**. coli DNA polymerase in the presence of 2 mM dNTPs.

DETD Vero cell monolayers were infected at an **m**.o.i. equal to 10 pfu/cell with parental or recombinant viruses. At 1 hr post-infection, the inoculum was aspirated and methionine-free medium. . .

DETD In order to determine whether the env gene product expressed by vCP83 and vCP87 was transported to the plasma **membrane** of infected cells, immunofluorescence experiments were performed as described previously (Taylor et al., 1990). Primary CEF monolayers were infected with. . .

DETD . . . to challenge with feline leukemia virus

		Time (weeks) relative to challenge						
	Cat	-5	-2	0	+3	+6	+9	+12
Group	No.	**E**.sup.1	V.sup.2					
			EV	EV	EV	F.sup.3	EV	
							FEV	FEV
1.	vCP 93:	1	--	--	--	++	-++	+++	+++
	Felv-A	2.	.	.	++	-++	+++	+++	
		17	--	--	--	++	-++	+++	+++
		18	--	--	--	++	-++	+++	+++

*__E__ = FeLV p27 antigen in plasma (ELISA)
V = infectious virus in plasma (virus isolation)
F = FeLV antigen in. . .

DETD The FHV-1 CO strain genomic DNA was completely digested with EcoRI and the fragment **M** (4470 bp) was excised from the agarose gel (Geneclean procedure) and cloned into vector pBS-SK+digested with EcoRI and phosphatased. The resulting plasmid containing the FHV-1 EcoRI **M** fragment was designated pHFeM2. The FHV-1 EcoRI **M** fragment complete nucleotide sequence for both strands was obtained from several subclones of the FHV-1 EcoRI **M** fragment inserted into vector pBS-SK.sup.+, using the modified T7 enzyme Sequenase (U.S. Biochemical Corp.) (Tabor and Richardson, 1987). Standard dideoxynucleotide. . .

DETD . . . the FHV-1 gD 5'-most region were confirmed by direct sequencing of pJCA071. Plasmid pJCA067 is a subclone of FHV-1 EcoRI **M** fragment. It has been generated as follows. Plasmid pHFeM2 was digested with BamHI and the 1850 bp BamHI-BamHI fragment was. . .

DETD Expression of the Hantaan virus G1 and G2 glycoproteins was accomplished by insertion of the **M** segment into the NYVAC and ALVAC vectors under the control of the entomopoxvirus 42 kDa promoter. The poxvirus expression cassette. . .

DETD A cDNA clone of the Hantaan virus **M** segment was derived as described by Schmaljohn et al. (1987) and provided by Dr. J. Dalrymple (Virology Division, U.S. Army. . . full sequence of the CDNA was presented previously by Schmaljohn et al. (1987). The 326 bp 5'-most region of the **M** segment coding sequence was derived using the plasmid pTZ19R containing the **M** segment cDNA as template and oligonucleotides HM5P (SEQ ID NO:335) (5'-ATGGGGA TATGGAAGTGG-3') and HM3P (SEQ ID NO:336) (5'-CATGTT CCTTTCAAGTCAAC-3'). This. . .

DETD The 3'-most 748 bp of the **M** segment coding sequence was derived by PCR using the cDNA clone contained in pTZ19R as template and oligonucleotides HMTS-5 (SEQ. . .

DETD The plasmid containing the **M**-specific cDNA clone in pTZ19R was used to transform GM48 (Dam.sup.-) bacterial cells (BRL, Gaithersburg, Md.). Plasmid DNA derived from this. . . the 42 kDa promoter fused to the

3' most region of the coding sequence. The resultant plasmid containing the entire **M** segment expression cassette was designated as pBSHVM. The entire **M** segment cassette was excised from pBSHVM using restriction endonucleases HindIII and EcoRI. The 3508 bp derived fragment was blunt-end using the Klenow fragment of the **E**. coli in the presence of 2 mM dNTPs. The blunt ended fragment was inserted into pSD550 to yield pHVMVC.

DETD . . . vP882. Recombinant virus was identified by in situ hybridization according to standard procedures (Piccini et al., 1987) using a radiolabeled **M**-specific DNA probe. Recombinant plaques were purified by 3 rounds of plaque purification and amplified for further analysis. Recombinant virus, vP882, contains the Hantaan **M** segment in the I4L locus of vaccinia virus. Replacement of the I4L open reading frame with the **M** segment cassette in the vP804 background creates a NYVAC- equivalent virus background (Tartaglia et al., 1992).

DETD The 3508 bp HindIII/EcoRI fragment derived from pBSHVM, containing the **M** segment cassette (above), was inserted into pC4I digested with HindIII and ZcoRI. The plasmid pC4I was derived as follows. A. . .

DETD Insertion of the **M** segment cassette into pC4I yielded plasmid pC4HVM. The plasmid pC4HVM was linearized with SmaI for insertion of a 100 bp. . . pC4HVMVQ was digested with SmaI followed by a subsequent partial HindIII digestion to recover a 3.6 kb fragment containing the **M** segment cassette. This fragment was blunt-ended using the klenow fragment of the **E**. coli DNA polymerase in the presence of 2mM dNTPs. This blunt-ended fragment was inserted into SmaI digested pSPCPC3L to generated. . .

DETD . . . identify and to purify the recombinant virus (as above; Piccini et al., 1987). The ALVAC-based recombinant containing the Hantaan virus **M** segment was designated as vCP114.

DETD . . . by linearization with XbaI followed by a partial HindIII digestion. This fragment was blunt-ended using the klenow fragment of the **E**. coli DNA polymerase in the presence of 2 mM dNTPs and then inserted into the SmaI site of pSD541 (defined. . .

DETD . . . in the ATI site and vP951 contains this cassette at the same locus, but by virtue of rescue with the **M** segment containing vP882, also contains the **M** segment in the 14L locus.

DETD The plasmid pBSHVM was linearized with SalI and blunt-ended using the Klenow fragment of the **E**. coli DNA polymerase in the presence of 2mM dNTPs. This was ligated to the 1.4 kb XbaI/partial HindIII (Blunt-ended with. . . from PBSHVS containing the Hantaan S segment expression cassette. The derived plasmid was designated as pBSHVMS. This plasmid contained the **M** and S cassettes in a head to head configuration. Plasmid pBSHVMS was linearized with XhoI, blunted with Klenow (as above),. . .

DETD . . . a 1.5 kb fragment containing the S segment expression cassette. This fragment was blunt-ended using the Klenow fragment of the **E**. coli DNA polymerase in the presence of 2 mM dNTPs and inserted into the SmaI site of pSPCP3L (defined in. . .

DETD Expression Analysis of the NYVAC- and ALVAC- Based Hantaan Virus **M** and S Segment Recombinants

DETD . . . Schmaljohn (Virology Division, U.S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, Frederick, Md.). The recombinant viruses containing the **M** segment alone (vP882, and VCP114) or in combination with the S segment (vP951) displayed intense surface fluorescence using any of. . .

DETD . . . inoculated with NYVAC-based Hepatitis B virus (HBV) recombinants vP856, vP930, vP932 and vP975 (Example 13). vP856 expresses spsAg, the middle (**M**) form of the surface antigen. vP930 expresses lpsAg, the large (L) form of the surface antigen. vP932 expresses both spsAg. . .

DETD TABLE 34

AUSAB and CORAB
Rabbits
Analysis of sera of rabbits inoculated with NYVAC-based HBV recombinants expressing

the middle (M) form of the surface antigen (small pre S antigen), the large (L) form
of the surface antigen (large pre S antigen) and. . . 2 regions fused to the core antigen.

week

#	vP HBV genes	1	2	3	4	5	6	7	8
AUSAB.sup.a									
A133	932								
	M + L >512								
		>512							
			262						
				352					
					13000				
						6500			
							3600		
								5400	
A134	932								
	M + L 250								
		235							
			72	80	3900				
						561			
							800		
								218	
A135	975								
	M + L + S/C								
		36	58	274.					
					406				
						1300			
							646		
								436	
									268
A136	975								
	M + L + S/C								
		103							
			>512						
				127					
					136				
						13468			
							4968		
								3168	
									2768
CORAB.sup.b									
A135	975								
	M + L + S/C								
		80	20	20	80	320	80	320	
								80	
A136	975								
	M + L + S/C								
		20	5	5	5	80	80	320	
								80	

Rabbits were inoculated with 10.sup.8 pfu of the. . .

DETD TABLE 35

Pre-S2 ELISA

Rabbits

Analysis by ELISA of sera from rabbits inoculated with NYVAC-based HBV recombinants expressing the middle (**M**) form of the surface antigen, the large (L) form of the surface antigen and a fusion protein (S/C) consisting of the pre. . . 2 regions fused to the core antigen.

week

#	vP HBV genes	1	2	3	4	5	6	7	8

A133 932
M + L 0 0 29 35 474
602
358
419
A134 932
M + L 0 0 0 277
2017
3099
847
500
A135 975
M + L + S/C
0 0 0 0 175
105
94 48
A136 975
M + L + S/C
0 0 0 0 2440
763
672
355

Rabbits were inoculated by the intramuscular (IM) route. . .

DETD TABLE 36

Pre-S1 ELISA

Rabbits

Analysis by ELISA test of sera from rabbits inoculated with NYVAC-based HBV recombinants expressing the middle (M) form and the large (L) form of the surface antigen and the preS1 + 2/core fusion protein (S/C).

#	vP	HBV genes	week 0	2	4	5	6	8
A133	932	M + L	<10	<10	<10	<10	<10	<10
A134	932	M + L	<10	<10	<10	<10	17	<10
A135	975	M + L + S/C	<10	<10	15	40	<10	24
A136	975	M + L + S/C	15	16	17	117	52	49

Rabbits were inoculated by the intradermal (ID) route with 10.sup.8. . .

DETD TABLE 37

Pre-S2 ELISA

Guinea Pigs

Analysis by ELISA of sera from guinea pigs inoculated with NYVAC-based HBV recombinants expressing the middle (M) form of the surface antigen, the large (L) form of the surface antigen and the preS1 + 2/core fusion protein (S/C).

#	VP	HBV genes	week 0	5	6
85	856	M	<10	<10	<10
86	856	M	<10	<10	<10
87	930	L	<10	46	35
88	930	L	<10	30	93
89	932	M + L	<10	39	<10
90	932	M + L	<10	33	19
91	975	M + L + S/C	<10	22	84
92	975	M + L + S/C	<10	53	269

Guinea pigs were inoculated by the SC route with 10.sup.8 of the

indicated. . .

DETD TABLE 38

CORAB
Mice
Analysis of sera by CORAB test of mice inoculated with vaccinia recombinant vP975 expressing the HBV middle (**M**) form of the surface antigen, the large (L) form of the surface antigen and a fusion protein (S/C) consisting of the pre. . . 2 regions fused to the core antigen.

Group	vP	HBV genes	Week 1	2	3	4	5	6	7	8
D	975	**M** + L + S/C	-.sup.a			-	-	-	5	5	5	5

Mice were inoculated by the IM route with. . .

DETD TABLE 39

Pre-S2 ELISA
Mice
Analysis by ELISA of sera from mice inoculated with NYVAC-based HBV recombinants expressing the middle (**M**) form of the surface antigen, the large (L) form of the surface antigen and the preS1 + 2/core fusion protein (S/C).

	vP	HBV genes	week 0	5	6
Group A	856	**M**	<10	73	70
Group B	930	L	<10	93	112
Group C	932	**M** + L	<10	970	1146
Group D	975	**M** + L + S/C	<10	1054	1062

Groups of eight or twelve mice were inoculated by the IM route with. . .

DETD TABLE 40

Pre-S2 ELISA
Mice
Analysis by ELISA of sera from mice inoculated with NYVAC-based HBV recombinants expressing the middle (**M**) form of the surface antigen, the large (L) form of the surface antigen and the preS1 + 2/core fusion protein (S/C).

	vP	HBV genes	week 0	5
Group B	930	L	60	244
Group C	932	**M** + L	66	125
Group D	975	**M** + L + S/C	63	1554

Groups of eight or twelve mice were inoculated by the IM route with 10.sup.7. . .

DETD . . . monolayers were either infected with parental virus, CPpp (ALVAC) or vP866 (NYVAC), or infected with vCP1661 or vP1075 at an **m**.o.i. of 10 pfu/cell. Cells were infected, incubated in modified Eagle's medium (minus methionine) containing [.sup.35 S]-methionine (20 .mu.Ci/ml), lysed and. . .

DETD . . . by Makoff et al., 1989) for fragment C produced by papain digestion of native tetanus toxin as well as an **E**. coli produced recombinant fragment C which is identical to that encoded by vCP161 and vP1075.

DETD . . . days post-challenge. NYVAC-based pseudorabies virus recombinant viruses were all shown to reduce the effects of the virulent pseudorabies virus challenge (i.**e**. clinical signs and virus isolation) compared to the controls, with the gp50 expressing recombinant virus being the most efficacious. In. . .

DETD . . . vP1015

A168	<1.3.sup.d						
		<1.3	<1.3	1.3.sup.c			
					2.2	2.2	2.2
A169	<1.3	1.6	1.6	1.6	3.1	3.1	2.5
Animals receiving vP913							
A116	<1.3	<1.3	.sup.	N.D..sup.**e**			
				1.3	2.8	2.2	2.2
A117	<1.3	<1.3	N.D.	<1.3	1.9	1.9	1.9

.sup.a Day of inoculation with 8.0 log.sub.10 pfu of. . . highest dilution showing a 50% reduction in plaque number as compared to preinoculation serum.

.sup.d Lowest dilution rested was 1:20

.sup.**e** Not done

DETD Construction of Insertion Vector Containing Japanese **Encephalitis Virus** (JEV) 15aaC, **prM**, **E**, NS21, NS2A

DETD . . . promoter, plasmid origin of replication and C5 flanking arms isolated. Plasmid JEVL14VC containing JEV cDNA encoding 15 amino acids C, **prM**, **E**, NS1, NS2A in a vaccinia virus donor plasmid (Mason et al., 1991) (nucleotides 337-4125, Konishi et al., 1991) was digested. .

DETD Construction of CS Insertion Vector Containing JEV 15aaC, **prM**, **E**

DETD . . . annealed oligonucleotides SP131 (SEQ ID NO:382) and SP132 (SEQ ID NO:383) (containing a SphI sticky end, T nucleotide completing the **E** coding region, translation stop, a vaccinia early transcription termination signal (AT5AT; Yuen and Moss, 1987), a second translation stop, and XbaI sticky end) generating plasmid JEVCP5 which encodes 15 amino acids C, **prM** and **E** under the control of the H6 promoter between C5 flanking arms.

DETD JEVCP1 was transfected into ALVAC infected primary CEF cells to generate the canarypox recombinant vCP107 encoding 15 amino acids C, **prM**, **E**, NS1, NS2A. JEVCP5 was transfected into ALVAC infected primary CEF cells to generate the canarypox recombinant vCP140 encoding JEV 15 aa C, **prM** and **E**. ##STR38##

DETD Immunoprecipitation experiments were performed as described previously (Konishi et al., 1991). The **E** protein produced in vCP107 and vCP140 infected cells comigrates with the **E** protein produced by JEV-vaccinia recombinants which have been shown to produce an authentic **E** protein (Konishi et al., 1991). vCP107 produces an NS1 protein that comigrates with the NS1 protein produced by JEV-vaccinia recombinants. . .

DETD TABLE 48

Protective efficacy of TROVAC-NDV (vFP96) in SPF and commercial broiler chickens.

Bird Group		NDV HI GMT.sup.d		Percent Protection.sup.**e**	
	Dose	Week 3	Week 4	NDV	FP
Group 1.sup.a					
	2.0	<5	<5	70	100
	4.0	<5	<5	70	100
	None. . . history of vaccination with fowlpox virus				

.sup.c : Specific pathogen free birds

.sup.d : Geometric mean titer of HI antibody

.sup.**e** : Percent protection of birds after NDV or Fowpox challenge

DETD . . . centrifugation and resuspended in Assay Medium (RPMI 1640 containing 10% fetal bovine serum, 20 mM HEPES, 2 mM L-glutamine, 5.times.10.sup.-5 **M** 2-mercaptoethanol, 100 U/ml penicillin, and 100 .mu.g/ml streptomycin). For memory CTL activity, the spleen cells from immunized mice were resuspended in Stimulation Medium (Minimum Essential Medium with Earle's salts containing 10% fetal bovine serum, 2mM L-glutamine, 10.sup.-4 **M** 2-mercaptoethanol, 100 U/ml penicillin, and 100 .mu.g/ml streptomycin) and stimulated in vitro in upright 25

cm.sup.2 tissue culture flasks with. . . titrated effector cells in 96-well microtiter plates for a 4 hr .sup.5 Cr release assay. Effector to target cell ratios (**E**:T) shown for the three assays were 100:1 (primary), 20:1 (memory), and 50:1 (secondary). Percent cytotoxicity was calculated as (experimental .sup.51. . .

DETD . . . and resuspended in the original volume of Cytotoxicity Medium, divided into two equal portions with or without complement (Rabbit Lo-Tox **M**, Cedarlane) and incubated at 37.degree. C for 45 min. The cells were then washed in Assay Medium and, based on. . .

DETD . . . apparent molecular masses of 160 kDa, 120 kDa, and 41 kDa, respectively. These are consistent with expression of the precursor **envelope** glycoprotein (160 kDa) and the proteolytically derived mature forms (120 kDa and 41 kDa).

DETD . . . digested pC5L to yield pC5HIV3BEEC. A 2.7 kb NruI/XbaI fragment from pBSHIV3BEECM was blunt-ended with the Klenow fragment of the **E**. coli DNA polymerase and inserted into NruI/SmaI digested pSPHAH6 to yield pHAHIV3BEEC.

DETD . . . by isolating the 2.1 kb NruI/XbaI fragment from PBSHIVMNT. This fragment was then blunt-ended with the Klenow fragment of the **E**. coli DNA polymerase in the presence of 2 mM dNTPs and inserted into pSPHAH6 digested with NruI and SmaI to. . .

DETD . . . Corp., Emeryville, Calif.). Investigation of surface immunofluorescence indicated that vCP138 and vP1035 infected cells contained HIV-1(MN) gp120 in the plasma **membrane**. Significantly, the surface staining of vCP138 and vP1035 infected cells was greatly enhanced compared to cells infected with recombinant viruses (i.**e**. vCP125, vCP124, vP1004, and vP1008) expressing gp160 or a non-anchored gp120. Results from immunoprecipitation analyses confirmed the expression of gp120. . .

DETD . . . precursor protein. Vero cell monolayers were either mock infected, infected with the parental virus or infected with vP969 at an **m**.o.i. of 10 PFU/cell. Following an hour adsorption period, the inoculum was aspirated and the cells were overlayed with 2 mls. . .

DETD An H6-promoted truncated HIV-1 **envelope** gene was then inserted into pHIVG4. This was accomplished by cloning the **E**. coli DNA polymerase I (Klenow fragment) filled-in 1,600 bp XhoI-NotI fragment of pHIVE10, containing an H6-promoted truncated HIV-1 **envelope** gene, into the filled-in BamHI site of pHIVG4. The plasmid generated by this manipulation is called pHIVGE11.

DETD . . . New Haven, Conn.). The plasmid pBSHIV3BCDT1 contains an H6 promoted cassette to express a severely truncated form of the HIV-1(IIIB) **envelope** (amino acid 1 to 447; Ratner et al., 1985). Expression of this cassette was evaluated to eliminate CD4 binding while. . .

DETD An H6-promoted truncated HIV-1 **envelope** gene was then inserted into pHIVG7. This was accomplished by cloning the **E**. coli DNA polymerase I (Klenow fragment) filled-in 1,600 bp XhoI-NotI fragment of pHIVE10 (defined in Example 95), containing an H6-promoted truncated HIV-1 **envelope** gene, into the filled-in BamHI site of pHIVG7. The plasmid generated by this manipulation is called pHIVGE12.

DETD The H6-promoted HIV-1(MN) **envelope** (gp120) gene was then inserted into pHIVGE14. This was accomplished by cloning the oligonucleotides, HIVL29 (SEQ ID NO:421) (5'-GGCCGCAAC-3') and. . .

DETD The H6-promoted **envelope** (gp120) gene and the I3L-promoted gag and pol genes were then inserted into a vaccinia virus insertion vector. This was. . .

DETD . . . by the gp160 gene. This was accomplished by cloning the 2,600 bp NruI-NotI fragment of pH6HMNE, containing the entire HIV-1(MN) **envelope** (gp160) gene, into the 8,000 bp partial NruI-NotI fragment of pHIVGE16. The plasmid generated by this manipulation is called pHIVGE19.

DETD . . . gene products. CEF cell monolayers were either mock infected, infected with the parental virus or infected with vCP117 at an **m**.o.i. of 10 PFU/cell. Following an hour adsorption period, the inoculum was aspirated and the cells were overlayed with 2 mls. . .

DETD . . . by the gp160 gene. This was accomplished by cloning the 2,600 bp NruI-NotI fragment of pH6HMNE, containing the entire HIV-1(MN)

envelope (gp160) gene, into the 9,000 bp NruI-NotI fragment of pHIVGE15. The plasmid generated by this manipulation is called pHIVGE18.

DETD . . . gene products. CEF cell monolayers were either mock infected, infected with the parental virus or infected with vCP130 at an **m**.o.i. of 10 PFU/cell. Following an hour adsorption period, the inoculum was aspirated and the cells were overlayed with 2 mls. . .

DETD . . . gag-pol and env genes would also produce such particles. Furthermore, if these ALVAC-based recombinants were used to infect non-avian cells (i.**e**. Vero, MRC-5, etc.) then HIV-1 virus-like particles could be purified without any poxvirus virion contaminants.

DETD . . . evaluate particle formation using Vero cells infected with vCP156, the following experiment was performed. Vero cells were infected at an **m**.o.i. of approximately 5 pfu/cell. After a 24 hr infection period, the supernatant was harvested and clarified by centrifugation at 2000. . . With the size exclusion noted above, the p24 would have passed through unless it was in a higher structural configuration (i.**e**. virus-like particles). Therefore, these results strongly suggest that HIV-1 virus-like particles containing the gp120 **envelope** component are produced in vCP156 infected cells.

DETD . . . gene products. Vero cell monolayers were either mock infected, infected with the parental virus or infected with vP1045 at an **m**.o.i. of 10 PFU/cell. Following an hour adsorption period, the inoculum was aspirated and the cells were overlayed with 2 mls. . .

DETD . . . gene products. CEF cell monolayers were either mock infected, infected with the parental virus or infected with vCP153 at an **m**.o.i. of 10 PFU/cell. Following an hour adsorption period, the inoculum was aspirated and the cells were overlayed with 2 mls. . .

DETD . . . precursor proteins. Vero cell monolayers were either mock infected, infected with the parental virus or infected with vP948 at an **m**.o.i. of 10 PFU/cell. Following an hour adsorption period, the inoculum was aspirated and the cells were overlayed with 2 mls. . .

DETD Macaque sera from SIV seropositive individuals specifically precipitated the SIV gag precursor protein and the **envelope** glycoprotein from vP948 infected cells, but did not precipitate SIV-specific proteins from mock infected cells.

DETD The plasmid, pSIVEMVC, contains the H6-promoted SIV.sub.MAC142 **envelope** gene (in vitro selected truncated version). The region of the **envelope** gene containing the premature termination codon was cloned into pBSK+. This was accomplished by cloning the 1,120 bp ClaI-BamHI fragment. . .

DETD At day 56 (i.**e**. 28 days after the second injection) protective titers were achieved in 0/3 of Group A, 2/3 of Group B and. . .

DETD . . . 35 56

1	10.sup.3.5					
		<0.1	<0.1	<0.1	<0.1	0.2
3	10.sup.3.5					
		<0.1	<0.1	<0.1	<0.1	<0.1
4	10.sup.3.5					
		<0.1	<0.1	<0.1	<0.1	<0.1
	G.**M**.T.	<0.1	<0.1	<0.1	<0.1	<0.1
6	10.sup.4.5					
		<0.1	<0.1	<0.1	<0.1	<0.1
7	10.sup.4.5					
		<0.1	<0.1	<0.1	2.4	1.9
10	10.sup.4.5					
		<0.1	<0.1	<0.1	1.6	1.1
	G.**M**.T.	<0.1	<0.1	0.1	0.58	0.47
11	10.sup.5.5					
		<0.1	<0.1	1.0	3.2	4.3
13	10.sup.5.5					
		<0.1	<0.1	0.3	6.0	8.8
14	10.sup.5.5					
		<0.1	<0.1.	. .	0.3	3.7
21	10.sup.5.5					
		<0.1	<0.1	0.2	2.6	3.9

25	10.sup.5.5					
		<0.1	<0.1	<0.1	1.7	4.2
25	10.sup.5.5					
		<0.1	<0.1	<0.1	0.6	0.9
	G.M.T.	<0.1	<0.1	0.16	1.9	4.4*
2	HDC	<0.1	<0.1	0.8	7.1	7.2
5	HDC	<0.1	<0.1	9.9	12.8	18.7
8	HDC	<0.1	<0.1.	. .	7.7	20.7
19	HDC	<0.1	<0.1	2.6	9.9	9.1
22	HDC	<0.1	<0.1	1.4	8.6	6.6
24	HDC	<0.1	<0.1	0.8	5.8	4.7
	G.M.T.	<0.1	<0.1	2.96	9.0	11.5*

*p = 0.007 student t test

DETD PROTECTION AGAINST JAPANESE **ENCEPHALITIS VIRUS** BY NYVAC-JEV RECOMBINANTS (vP908, vP923)

DETD Using NYVAC-JEV recombinants, protection against Japanese **Encephalitis virus** was provided. NYVAC vP866, NYVAC recombinants vP908 and vP923, and vaccinia recombinants vP555 and vP829 were produced as described herein.

DETD . . . positioned behind the early/late H6 promoter. Recombinant vP908 (and vP555; Mason et al., 1991) includes the putative 15 amino acid **signal sequence** preceding the N-terminus of **prM**, **prM**, **E**, NS1 and NS2A. Recombinant vP923 (and vP828; Konishi et al., 1991) encodes the putative **signal sequence** of **prM**, **prM**, and **E**.

DETD Synthesis of **E** and NS1 by Recombinant Vaccinia Viruses. Immunoprecipitation of the **E** or NS1 gene was performed using a monoclonal antibody specific for **E** or NS1. Proteins reactive with the **E** MAb were synthesized in cells infected with vP555, vP908 and vP923, and proteins reactive with NS1 MAb were synthesized in. . . cells infected with vP555 and vP908 but not in cells infected with vP923. vP555 infected cells produced correct forms of **E** and NS1 inside and outside of the cell. The proteins produced by vP908 and vP923 were identical in size to those produced by vP555. For both **E** and NS1, the extracellular forms migrated slower than the intracellular forms in SDS-PAGE, consistent with maturation of the N-linked glycans. . . the JEV genome (Mason et al., 1987). Immunoprecipitates prepared from radiolabeled vaccinia recombinant infected cells using a MAb specific for **M** (and **prM**) revealed that **prM** was synthesized in cells infected with vP908 and vP923.

DETD The immune response to **E** correlated well with the results of the NEUT and HAI tests. The RIP response to **E** observed in swine immunized with vP923 on day 35 was higher than the RIP response to **E** in swine immunized with vP908, whereas the HAI titers on day 35 were equivalent in the two groups. However, NEUT. . . be induced but the quantitative aspects of the RIP analysis was not further validated. Weak RIP responses of sera to **E** on day seven in spite of relatively high NEUT antibody titers could be explained by IgM antibody early after immunization.. . .

DETD . . . sera collected 20 days post-challenge for antibodies against JEV. The swine vaccinated with vP908 or vP923 had higher responses to **E** than those inoculated with PBS or vP866, indicating that the antibody reactivity to **E** that was present before challenge was boosted by JEV infection. Reactions to NS3 and NS5, JEV proteins which were not. . .

DETD TABLE 52

Immunization and JEV challenge in mice

Immunizing Virus.sup.a	JEV Genes Expressed	Antibody titer NEUT.sup.b	HAI.sup.c	Survival.sup.d
vP829	**prM, E**	1:320	1:80	10/10 (100%)

vP908	**prM, E,** NS	1:320	1:80	11/12 (92%)
vP923	**prM, E**	1:320	1:80	12/12 (100%)

.sup.a Vaccinia recombinant virus used for immunizing groups of 4week old mice.

.sup.b Serum dilution yielding. . .

DETD . . . recombinants have also been shown to elicit measles virus neutralizing antibodies in rabbits and protection against pseudorabies virus and Japanese **encephalitis virus** challenge in swine. The highly attenuated NYVAC strain confers safety advantages with human and veterinary applications (Tartaglia et al., 1990).. . .

DETD . . . skin); c) absence of testicular inflammation (nude mice); d) greatly reduced virulence (intracranial challenge, both three-week old and newborn mice); **e**) greatly reduced pathogenicity and failure to disseminate in immunodeficient subjects (nude and cyclophosphamide treated mice); and f) dramatically reduced ability. . .

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. . . in claim 5 wherein the exogenous DNA is selected from the group consisting of rabies virus, Hepatitis B virus, Japanese **encephalitis virus**, **yellow fever virus**, **Dengue** virus, measles virus, pseudorabies virus, Epstein-Barr virus, herpes simplex virus, human immunodeficiency virus, simian immunodeficiency virus, equine herpes virus, bovine. . .

. . . 20. A virus as claimed in claim 6 wherein the virus is a canarypox virus, the non-poxvirus source is Japanese **encephalitis virus** and the canarypox virus is vCP107 or vCP140.

L14 ANSWER 12 OF 15 USPATFULL on STN

1998:44886 **Flavivirus** recombinant poxvirus immunological composition.

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US 5744141 19980428

APPLICATION: US 1995-484304 19950607 (8) <--

DOCUMENT TYPE: Utility; Granted.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB What is described is a recombinant poxvirus, such as vaccinia virus, fowlpox virus and canarypox virus, containing foreign DNA from **flavivirus**, such as Japanese **encephalitis virus**, **yellow fever virus** and **Dengue** virus. In a preferred embodiment, the recombinant poxvirus generates an extracellular particle containing **flavivirus E** and **M** proteins capable of inducing neutralizing antibodies, hemagglutination-inhibiting antibodies and protective immunity against **flavivirus** infection. What is also described is a vaccine containing the recombinant poxvirus for inducing an immunological response in a host animal inoculated with the vaccine.

CLM What is claimed is:

1. A recombinant poxvirus comprising DNA coding for at least one **flavivirus** structural protein, wherein the **flavivirus** is **Yellow Fever virus** or **Dengue** virus and the poxvirus is selected from the group consisting of: an avipox virus, a vaccinia virus wherein the open reading frames for the thymidine kinase gene, a hemorrhagic region, an A type inclusion body region, a hemagglutinin gene, a host range gene region and a large subunit, ribonucleotide reductase have been deleted therefrom, a vaccinia virus wherein regions C7L-K1L, J2R, B13R+B14R, A26L, A56R, and I4L have been deleted therefrom, and a NYVAC vaccinia virus.

2. The recombinant poxvirus of claim 1 wherein the DNA comprises a part of the **flavivirus** open reading frame from c to NS2b.

3. The recombinant poxvirus of claim 1 wherein the DNA encodes protein **M** or a precursor to protein **M**, and **flavivirus** proteins **E**, NS1 and NS2A.

4. The recombinant poxvirus of claim 1 wherein the poxvirus is a vaccinia virus.

5. The recombinant poxvirus of claim 1 wherein the poxvirus is an avipox virus.

6. The recombinant poxvirus of claim 5 wherein the avipox virus is canarypox virus.

7. The recombinant poxvirus of claim 1 wherein the **flavivirus** is

yellow fever virus.

8. The recombinant poxvirus of claim 1 wherein the **flavivirus** is **Dengue** virus.

9. The recombinant poxvirus of claim 6 wherein the canarypox virus is an ALVAC canarypox virus.

10. The recombinant poxvirus of claim 6 wherein the canarypox virus is attenuated through more than 200 serial passages on chick embryo fibroblasts, a master seed therefrom was subjected to four successive plaque purifications under agar, from which a plaque clone was amplified through five additional passages.

11. The recombinant poxvirus of claim 4 wherein in the vaccinia virus, the open reading frames for the thymidine kinase gene, a hemorrhagic region, an A type inclusion body region, a hemagglutinin gene, a host range gene region and a large subunit, ribonucleotide reductase have been deleted therefrom, or regions C7L-K1L, J2R, B13R+B14R, A26L, A56R, and I4L have been deleted therefrom.

12. The recombinant poxvirus of claim 4 wherein the vaccinia virus is a NYVAC vaccinia virus.

13. The recombinant poxvirus of claim 1 which is vCP127 or vCP107.

14. The recombinant poxvirus of claim 1 wherein the DNA comprises DNA encoding C-terminal amino acids of C.

15. The recombinant poxvirus of claim 1 wherein the DNA further comprises DNA encoding NS2b.

16. An immunological composition comprising a carrier and a recombinant poxvirus according to any one of claims 1-15, wherein the composition is effective to induce an immunological response in a a host.

17. A method for producing a **flavivirus** structural protein comprising introducing into a cell a recombinant poxvirus, transforming cell with the expression vector, cultivating the transformed cell under conditions which allow expression of the recombinant poxvirus, and further purifying the protein as claim in any one of claims 1-15.

TI **Flavivirus** recombinant poxvirus immunological composition

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AB What is described is a recombinant poxvirus, such as vaccinia virus, fowlpox virus and canarypox virus, containing foreign DNA from **flavivirus**, such as Japanese **encephalitis virus, yellow fever virus** and **Dengue** virus. In a preferred embodiment, the recombinant poxvirus generates an extracellular particle containing **flavivirus E** and **M** proteins capable of inducing neutralizing antibodies, hemagglutination-inhibiting antibodies and protective immunity against **flavivirus** infection. What is also described is a vaccine containing the recombinant poxvirus for inducing an immunological response in a host. . .

SUMM . . . and using the same. More in particular, the invention relates to recombinant poxvirus, which virus expresses gene products of a **flavivirus** gene, and to vaccines which provide protective immunity against **flavivirus** infections.

SUMM . . . sequence to be inserted into the virus, particularly an open reading frame from a non-pox source, is placed into an **E**. coli plasmid construct into which DNA homologous to a section of DNA of the poxvirus has been inserted.

SUMM . . . flanking a region of pox DNA containing a nonessential locus. The resulting plasmid construct is then amplified by growth within **E**. coli bacteria (Clewell, 1972) and isolated (Clewell et al., 1969; Maniatis et al., 1986).

SUMM second, the isolated plasmid containing the DNA gene sequence to be inserted is transfected into a cell culture, **e**.g. chick embryo fibroblasts, along with the poxvirus. Recombination between homologous pox DNA in the plasmid and the viral genome respectively. . .

SUMM The family **Flaviviridae** comprises approximately 60 arthropod-borne viruses that cause significant public health problems in both temperate and tropical regions of the world. . . developed against some of these agents, there has been a recent surge in the study of the molecular biology of **flaviviruses** in order to produce recombinant vaccines to the remaining viruses, most notably **dengue** (Brandt, 1988).

SUMM **Flavivirus** proteins are encoded by a single long translational open reading frame (ORF) present in the positive-strand genomic RNA. The genes. . . end of the genome followed by the nonstructural glycoprotein NS1 and the remaining nonstructural proteins (Rice et al., 1985). The **flavivirus** virion contains an **envelope** glycoprotein, **E**, a **membrane** protein, **M**, and a capsid protein, C. In the case of Japanese **encephalitis virus** (JEV), virion preparations usually contain a small amount of the glycoprotein precursor to the **membrane** protein, **prM** (Mason et al., 1987a). Within JEV-infected cells, on the other hand, the **M** protein is present almost exclusively as the higher molecular weight **prM** protein (Mason et al., 1987a; Shapiro et al., 1972).

SUMM Studies that have examined the protective effect of passively administered monoclonal antibodies (MAbs) specific for each of the three **flavivirus** glycoproteins (**prM**, **E**, NS1) have demonstrated that immunity to each of these antigens results in partial or complete protection from lethal viral challenge. Monoclonal antibodies to **E** can provide protection from infection by Japanese **encephalitis virus** (JEV) (Kimura-Kuroda et al., 1988; Mason et al., 1989), **dengue** type 2 virus (Kaufman et al., 1987) and **yellow fever virus** (YF) (Gould et al., 1986). In most cases, passive protection has been correlated with the ability of these **E** MAbs to neutralize the virus in vitro. Recently, Kaufman et al. (1989) have demonstrated that passive protection can also be produced with **prM** MAbs that exhibit weak or undetectable neutralizing activity in vitro. The ability of structural protein specific MAbs to protect animals. . . attenuate viral infection by blocking virus binding to target cells. Passive protection experiments using MAbs to the NS1 protein of **yellow fever virus** (Schlesinger et al., 1985; Gould et al., 1986) and **dengue** type 2 virus (Henchal et al., 1988) have demonstrated that antibodies to this nonstructural glycoprotein can protect animals from lethal. . .

SUMM . . . of NS1 immunity to protect the host from infection comes from direct immunization experiments in which NS1 purified from either **yellow fever virus**-infected cells (Schlesinger et al., 1985, 1986) or **dengue** type 2 virus-infected cells (Schlesinger et al., 1987) induced protective immunity from infection with the homologous virus.

SUMM Although significant progress has been made in deriving the primary structure of these three **flavivirus** glycoprotein antigens, less is known about their three-dimensional structure. The ability to produce properly folded, and possibly correctly assembled, forms. . . NS1-based vaccines, dimerization of NS1 (Winkler et al., 1988) may be required to elicit the maximum protective response. For the **E** protein, correct: folding is probably required for eliciting a protective immune response since **E** protein antigens produced in **E**. coli (Mason et al., 1989) and the authentic **E** protein prepared under denaturing conditions (Wengler et al., 1989b) failed to induce neutralizing antibodies. Correct folding of the **E** protein may require the coordinated synthesis of the **prM** protein, since these proteins are found in heterodimers in the cell-associated forms of West Nile virus (Wengler et al., 1989a). The proper folding of **E** and the assembly of **E** and **prM** into viral particles may require the coordinated synthesis of the NS1 protein, which is coretained in an early compartment of the secretory apparatus along with immature forms of **E** in JEV-infected cells (Mason, 1989).

SUMM Attempts to produce recombinant **flavivirus** vaccines based on the **flavivirus** glycoproteins has met with some success, although protection in animal model systems has not always correlated with the

predicted production. . .

SUMM . . . a vaccinia recombinant containing the region of JEV encoding 65 out of the 127 amino acids of C, all of **prM**, all of **E**, and 59 out of the 352 amino acids of NS1. Haishi et al. (1989) reported a vaccinia recombinant containing Japanese encephalitis sequences encoding 17 out of the 167 amino acids of **prM**, all of **E** and 57 out of the 352 amino acids of NS1.

SUMM Deubel et al. (1988) reported a vaccinia recombinant containing the **dengue**-2 coding sequences for all of C, all of **prM**, all of **E** and 16 out of the 352 amino acids of NS1.

SUMM Zhao et al. (1987) reported a vaccinia recombinant containing the **dengue**-4 coding sequences for all of C, all of **prM**, all of **E**, all of NS1, and all of NS2A. Bray et al. (1989) reported a series of vaccinia recombinants containing the **dengue**-4 coding sequences for (i) all of C, all of **prM** and 416 out of the 454 amino acids of **E**, (ii) 15 out of the 167 amino acids of **prM** and 416 out of the 454 amino acids of **E**, (iii) 18 amino acids of influenza A virus hemagglutinin and 416 out of the 454 amino acids of **E**, and (iv) 71 amino acids of respiratory syncytial virus G glycoprotein and 416 out of the 454 amino acids of **E**.

SUMM Despite these attempts to produce recombinant **flavivirus** vaccines, the proper expression of the JEV **E** protein by the vaccinia recombinants has not been satisfactorily obtained. Although Haishi et al. (1989) demonstrated cytoplasmic expression of JEV **E** protein by their vaccinia recombinant, the distribution was different from that observed in JEV infected cells. Yasuda et al. (1990) detected expression of JEV **E** protein by their vaccinia recombinant on the cell surface. Recombinant viruses that express the **prM** and **E** protein protected mice from approximately 10 LD_{50} of challenge virus. Yasuda et al. (1990) elicited anti-JEV immune responses as well as protection but reactivity to a panel of **E** specific monoclonal antibodies exhibited differences from the reactivity observed in JEV infected cells.

SUMM **Dengue** type 2 structural proteins have been expressed by recombinant vaccinia viruses (Deubel et al., 1988). Although these viruses induced the synthesis of the structural glycoprotein within infected cells, they neither elicited detectable anti-**dengue** immune responses nor protected monkeys from **dengue** infection. Several studies also have been completed on the expression of portions of the **dengue** type 4 structural and nonstructural proteins in vaccinia virus (Bray et al., 1989; Falgout et al., 1989; Zhao et al.,. . . the viral ORF extending from C to NS2A under the control of the P7.5 early-late promoter produced intracellular forms of **prM**, **E**, and NS1 but failed to induce the synthesis of extracellular forms of any of the structural proteins, even though a. . . this recombinant virus (Bray et al., 1989; Zhao et al., 1987). Additional recombinant viruses that contained several forms of the **dengue** type 4 **E** gene with or without other structural protein genes have also been examined (Bray et al., 1989). Although several of these recombinant viruses were able to induce protection, they neither produced extracellular forms of **E** nor induced neutralizing antibodies. A **dengue**-vaccinia recombinant expressing a C-terminally truncated **E** protein gene induced the synthesis of an extracellular form of **E** and provided an increasing level of resistance to **dengue** virus encephalitis in inoculated mice (Men et al., 1991).

SUMM It can thus be appreciated that provision of a **flavivirus** recombinant poxvirus which produces properly processed forms of **flavivirus** proteins, and of vaccines which provide protective immunity against **flavivirus** infections, would be a highly desirable advance over the current state of technology.

SUMM It is therefore an object of this invention to provide recombinant poxviruses, which viruses express properly processed gene products of **flavivirus**, and to provide a method of making such recombinant poxviruses.

SUMM It is an additional object of this invention to provide for the cloning and expression of **flavivirus** coding sequences in a poxvirus vector.

SUMM It is another object of this invention to provide a vaccine which is capable of eliciting **flavivirus** neutralizing antibodies,

hemagglutination inhibiting antibodies and protective immunity against **flavivirus** infection and a lethal **flavivirus** challenge.

SUMM In one aspect, the present invention relates to a recombinant poxvirus generating an extracellular **flavivirus** structural protein capable of inducing protective immunity against **flavivirus** infection. In particular, the recombinant poxvirus generates an extracellular particle containing **flavivirus E** and **M** proteins capable of eliciting neutralizing antibodies and hemagglutination-inhibiting antibodies. The poxvirus is advantageously a vaccinia virus or an avipox virus, such as fowlpox virus or canarypox virus. The **flavivirus** is advantageously Japanese **encephalitis virus**, **yellow fever virus** and **Dengue** virus.

SUMM According to the present invention, the recombinant poxvirus contains therein DNA from **flavivirus** in a nonessential region of the poxvirus genome for expressing in a host **flavivirus** structural protein capable of release to an extracellular medium. In particular, the DNA contains Japanese **encephalitis virus** coding sequences that encode a precursor to structural protein **M**, structural protein **E**, and nonstructural proteins NS1 and NS2A. More in particular, the recombinant poxvirus contains therein DNA from **flavivirus** in a nonessential region of the poxvirus genome for expressing a particle containing **flavivirus** structural protein **E** and structural protein **M**.

SUMM . . . with the vaccine, said vaccine including a carrier and a recombinant poxvirus containing, in a nonessential region thereof, DNA from **flavivirus**.

SUMM More in particular, the recombinant viruses express portions of the **flavivirus** ORF extending from **prM** to NS2B. Biochemical analysis of cells infected with the recombinant viruses showed that the recombinant viruses specify the production of properly processed forms of all three **flavivirus** glycoproteins--**prM**, **E**, and NS1. The recombinant viruses induced synthesis of extracellular particles that contained fully processed forms of the **M** and **E** proteins. Furthermore, the results of mouse immunization studies demonstrated that the induction of neutralizing antibodies and high levels of protection were associated with the ability of the immunizing recombinant viruses to produce extracellular particles containing the two structural **membrane** proteins.

DRWD FIG. 7 shows a comparison by SDS-PAGE analysis of the cell lysate **E** proteins produced by JEV infection and infection with the recombinant vaccinia viruses vP650, vP555, vP658 and vP583;

DRWD FIG. 8 shows a comparison by SDS-PAGE analysis of the culture fluid **E** proteins produced by JEV infection and infection with the recombinant vaccinia viruses vP650, vP555, vP658 and vP583;

DRWD FIG. 9 shows a comparison by sucrose gradient analysis of the forms of the **E** protein found in the culture fluid harvested from JEV infected cells and cells infected with vaccinia recombinants vP555 and vP650;

DETD . . . and B (SEQ ID NO:52) which contains the sequence of the C coding region combined with an updated sequence of **prM**, **E**, NS1, NS2A and NS2B coding regions.

DETD . . . The resulting plasmid, pJEV1, contained the viral ORF extending from the SacI site (nucleotide 2125) in the last third of **E** through the BalI site (nucleotide 4125) two amino acid residues (aa) into the predicted N terminus of NS2B (FIG. 1).

DETD . . . containing a XhoI sticky end, a SmaI site, the last 15 aa of C, and first 9 aa of JEV **prM** with a sticky HindIII end) were ligated to a HindIII-SacI fragment of JEV CDNA (nucleotides 407-2124), and XhoI-SacI digested vector. . . the viral ORF extending between the methionine (Met) codon (nucleotides 337-339) occurring 15 aa preceding the predicted N terminus of **prM** and the SacI site (nucleotide 2124) found in the last third of **E** (FIG. 1).

DETD . . . pJEV5, contained the viral ORF extending between the Met codon (nucleotides 811-813) occurring 25 aa preceding the N terminus of **E** and the SacI site (nucleotide 2124) found in the last third of **E** (FIG. 1).

DETD . . . (Kunkel, 1985) was used to change a potential vaccinia virus early transcription termination signal (Yuen et al., 1987) in the **E** gene of pJEV2 (TTTTTGT; nucleotides 1304-1310) to TCTTTGT, creating plasmid pJEV22 (FIG. 2). The same change was performed on pJEV5. . .

DETD . . . resulting plasmid, pBEV7, contained the viral ORF extending between the SacI site (nucleotide 2125) found in the last third of **E** and the last codon of NS2B (nucleotide 4512) (FIG. 2). SmaI-EagI digested pTP15 was purified and ligated to the purified. . .

DETD BHK or VERO cell monolayers were prepared in 35 mm diameter dishes and infected with vaccinia viruses (**m**.o.i. of 2) or JEV (**m**.o.i. of 5) and incubated for 11 hr (vaccinia) or 16 hr (JEV) before radiolabeling. At 11 hr or 16 hr. . .

DETD Four different vaccinia virus recombinants were constructed that expressed portions of the JEV coding region extending from **prM** through NS2B. The JEV cDNA sequences contained in these recombinant viruses are shown in FIG. 4. In all four recombinant. . .

DETD Recombinant vP555 encodes the putative 15 aa **signal sequence** preceding the N terminus of the structural protein precursor **prM**, the structural glycoprotein **E**, the nonstructural glycoprotein NS1, and the nonstructural protein NS2A (McAda et al., 1987). Recombinant vP583 encodes the putative **signal sequence** preceding the N terminus of **E**, **E**, NS1, and NS2A (McAda et al., 1987). Recombinant vP650 contains a cDNA encoding the same proteins as vP555 with the. . . vP583 with the addition of NS2B. In recombinants vP650 and vP658, a potential vaccinia virus early transcription termination signal in **E** (TTTTTGT; nucleotides 1087-1094) was modified to TCTTTGT without altering the aa sequence. This change was made in an attempt to increase the level of expression of **E** and NS1, since this sequence has been shown to increase transcription termination in in vitro transcription assays (Yuen et al.,. . .

DETD . . . noted that recombinants vP555, vP583, and vP650 had a deletion from within the HindIII C fragment through HindIII N and **M** and into HindIII K. This same deletion was observed in the vP425 parental virus. Interestingly, these viruses were less cytopathic. . .

DETD . . . lysate (FIG. 5) or culture fluid (FIG. 6) prepared from each cell layer were immunoprecipitated, and then either mock digested (**M**), digested with endo H (H), or digested with PNGase F (F), prior to SDS-PAGE analysis.

DETD . . . production by all four recombinants, suggesting that the potential vaccinia early transcriptional termination signal present near the end of the **E** coding region in vP555 and vP583 did not significantly reduce the amount of NS1 produced relative to vP650 or vP658. . .

DETD **E** and **prM** were Properly Processed when Expressed by Recombinant Vaccinia Viruses

DETD FIGS. 7 and 8 show a comparison of the **E** protein produced by JEV infection or infection with the recombinant vaccinia viruses. BHK cells were infected with JEV or recombinant. . . lysate (FIG. 7) or culture fluid (FIG. 8) prepared from each cell layer were immunoprecipitated, and then either mock digested (**M**), digested with endo H (H), or digested with PNGase F (F), prior to SDS-PAGE analysis.

DETD The data from the pulse-chase experiments depicted in FIGS. 7 and 8 demonstrate that proteins identical in size to **E** were synthesized in cells infected with all recombinant vaccinia viruses containing the **E** gene. However, the **E** protein was only released from cells infected with vaccinia viruses that contained the region of the viral ORF encoding **prM**, **E**, NS1, and NS2A (vP555 and vP650; see FIGS. 4, 7 and 8). Endoglycosidase sensitivity (FIGS. 7 and 8) revealed that both the intracellular and extracellular forms of the **E** protein synthesized by cells infected with the vaccinia recombinants were glycosylated; the cell-associated forms of **E** were endo H sensitive, whereas the extracellular forms were resistant to endo H digestion.

DETD Immunoprecipitates prepared from radiolabeled vaccinia-infected cells using a MAb specific for **M** (and **prM**) revealed that **prM** was synthesized in cells infected with vP555 and vP650. Cells infected with either of these recombinant vaccinia viruses produced cellular forms of **prM** that were identical in size to the **prM** protein produced by JEV-infected cells, and a **M** protein of the correct size was detected in the culture fluid of cells infected with these two viruses.

DETD The extracellular fluid harvested from cells infected with vP555 and

vP650 contained forms of **E** that migrated with a peak of hemagglutinating activity in sucrose density gradients. Interestingly, this hemagglutinin migrated similarly to the slowly. . . found in the culture fluid of JEV-infected cells (FIG. 9). Furthermore, these same fractions contained the fully processed form of **M**, demonstrating that vP555- and vP650-infected cells produced a particle that contained both of the structural **membrane** proteins of JEV. These particles probably represent empty JEV envelopes, analogous to the 22 nm hepatitis B virus particles found. . .

DETD Recombinant vaccinia virus vP555 produced **E**- and **M**-containing extracellular particles that behaved like empty viral envelopes. The ability of this recombinant virus to induce the synthesis of extracellular. . .

DETD The recombinant viruses described herein contain portions of the JEV ORF that encode the precursor to the structural protein **M**, the structural protein **E**, and nonstructural proteins NS1, NS2A, and NS2B. The **E** and NS1 proteins produced by cells infected with these recombinant viruses underwent proteolytic cleavage and N-linked carbohydrate addition in a. . . proteins produced by cells infected with JEV. These data further demonstrate that the proteolytic cleavage and N-linked carbohydrate addition to **E** and NS1 do not require **flavivirus** nonstructural proteins located 3' to NS2A in the viral genome (Bray et al., 1989; Deubel et al., 1988; Falgout et. . .

DETD . . . the portion of the ORF inserted in the recombinant vaccinia viruses had a significant effect on the late-stage processing of **prM** and **E**, but not on the fate of NS1. All recombinant viruses that encoded NS1 produced mature extracellular forms of this protein,. . . from transfected cells (Fan et al., 1990). On the other hand, only two of the four recombinants that contained the **E** protein coding region produced extracellular forms of **E**. These two recombinants, vP555 and vP650, differed from the remaining recombinants in that they contained the **prM** coding region in addition to **E**, NS1, and NS2A. The findings that extracellular forms of **E** were produced only by viruses containing the coding regions for both **E** and **prM** and that the extracellular forms of **E** were associated with **M** suggest that the simultaneous synthesis of **prM** and **E** is a requirement for the formation of particles that are targeted for the extracellular fluid.

DETD . . . the co-migration of the rabbit immunoglobulin heavy chain with the radiolabeled viral antigens, and to permit clear separation of the **E** and the NS1' proteins. Neutralization tests were performed on heat-inactivated sera (20 min. at 56° C.) as described (Tesh et. . .

DETD . . . virus, two viruses (vP555 and vP658) were selected for in-depth challenge studies. vP555 induced the synthesis of extracellular forms of **E**, whereas vP658 did not produce any extracellular forms of **E**, but contained additional cDNA sequences encoding the NS2B protein. In the challenge experiments several dilutions of challenge virus were tested,. . . dose of JEV. The analysis demonstrated that: (1) only those animals immunized with vP555 showed a strong immune response to **E**, and (2) a second inoculation resulted in a significant increase in reactivity to the **E** protein (FIG. 10).

DETD . . . induce neutralizing antibodies may be related to the fact that vP555 produces an extracellular particulate form of the structural proteins **E** and **M**. This SHA-like particle probably represents an empty JEV **envelope** that contains **E** and **M** folded and assembled into a configuration very similar to that found in the infectious JEV particle. Recombinants vP555 and vP650. . . assembly of viral envelopes. Other investigators (see above) have not been able to detect the production of extracellular forms of **E** by cells expressing all three structural proteins (C, **prM**, and **E**) in the presence or absence of NS1 and NS2A. The inability of their recombinant viruses to produce particles similar to. . . that the C protein produced in the absence of a genomic RNA interferes with the proper assembly of the viral **membrane** proteins. Alternatively, an incompletely processed form of C similar to that detected by Nowak et al. (1989) in in vitro translation experiments, could prevent release of the structural **membrane** proteins

from the cells expressing the C gene.

DETD . . . were obtained from GIBCO/BRL, Gaithersburg, MD, New England Biolabs, Beverly, MA; and Boehringer Mannheim Biochemicals, Indianapolis, IN. Klenow fragment of **E**. coli polymerase was obtained from Boehringer Mannheim Biochemicals. BAL-31 exonuclease and phage T4 DNA ligase were obtained from New England. . .

DETD . . . NcoI site (pos. 172,253) were removed by digestion of pSD419 with NcoI/SmaI followed by blunt ending with Klenow fragment of **E**. coli polymerase and ligation generating plasmid pSD476. A vaccinia right flanking arm was obtained by digestion of pSD422 with HpaI. . . ID NO:6/SEQ ID NO:7) ##STR3## generating pSD479. pSD479 contains an initiation codon (underlined) followed by a BamHI site. To place **E**. coli Beta-galactosidase in the B13-B14 (u) deletion locus under the control of the u promoter, a 3.2 kb BamHI fragment. . .

DETD . . . at the pUC/vaccinia junction was destroyed by digestion of pSD478 with EcoRI followed by blunt ending with Klenow fragment of **E**. coli polymerase and ligation, generating plasmid pSD478E$^-$. pSD478E$^-$ was digested with BamHI and HpaI and ligated with annealed synthetic oligonucleotides. . .

DETD . . . XbaI within vaccinia sequences (pos. 137,079) and with HindIII at the pUC/vaccinia junction, then blunt ended with Klenow fragment of **E**. coli polymerase and ligated, resulting in plasmid pSD483. To remove unwanted vaccinia DNA sequences to the right of the A26L. . . digestion with BglII (pos. 140,136) and with EcoRI at the pUC/vaccinia junction, followed by blunt ending with Klenow fragment of **E**. coli polymerase and ligation. The resulting plasmid was designated pSD489. The 1.8 kb ClaI (pos. 137,198)/EcoRV (pos. 139,048) fragment from. . .

DETD A 3.3 kb BglII cassette containing the **E**. coli Beta-galactosidase gene (Shapira et al., 1983) under the control of the vaccinia 11 kDa promoter (Bertholet et al., 1985;. . .

DETD A 3.2 kb BglII/BamHI (partial) cassette containing the **E**. coli Beta-galactosidase gene (Shapira et al., 1983) under the control of the vaccinia 11 kDa promoter (Bertholet et al., 1985;. . .

DETD . . . were removed from the pUC/vaccinia junction by digestion of pSD466 with EcoRI/BamHI followed by blunt ending with Klenow fragment of **E**. coli polymerase and ligation. Recombination between vP708 and pSD467 resulted in recombinant: vaccinia deletion mutant, vP723, which was isolated as. . .

DETD . . . SphI and religated, forming pSD451. In pSD451, DNA sequences to the left of the SphI site (pos. 27,416) in HindIII **M** are removed (Perkus et al., 1990). pSD409 is HindIII **M** cloned into pUC8.

DETD To provide a substrate for the deletion of the [C7L-K1L] gene cluster from vaccinia, **E**. coli Beta-galactosidase was first inserted into the vaccinia M2L deletion locus (Guo et al., 1990) as follows. To eliminate the. . . unique BglII site inserted into the M2L deletion locus as indicated above. A 3.2 kb BamHI (partial)/BglII cassette containing the **E**. coli Beta-galactosidase gene (Shapira et al., 1983) under the control of the 11 kDa promoter (Bertholet et al., 1985) was. . .

DETD . . . deleted for vaccinia genes [C7L-K1L] was assembled in PUCS cut with SmaI, HindIII and blunt ended with Klenow fragment of **E**. coli polymerase. The left flanking arm consisting of vaccinia HindIII C sequences was obtained by digestion of pSD420 with XbaI (pos. 18,628) followed by blunt ending with Klenow fragment of **E**. coli polymerase and digestion with BglII (pos. 19,706). The right flanking arm consisting of vaccinia HindIII K sequences was obtained. . .

DETD . . . coding sequences, pSD518 was digested with BamHI (pos. 65,381) and HpaI (pos. 67,001) and blunt ended using Klenow fragment of **E**. coli polymerase. This 4.8 kb vector fragment was ligated with a 3.2 kb SmaI cassette containing the **E**. coli Beta-galactosidase gene (Shapira et al., 1983) under the control of the vaccinia 11 kDa promoter (Bertholet et al., 1985;. . .

DETD . . . mutagenized expression cassette contained within pRW837 was derived by digestion with HindIII and EcoRI, blunt-ended using the Klenow fragment of **E**. coli DNA polymerase in the presence of 2mM dNTPs, and inserted into the SmaI site of pSD513 to yield pRW843.. . .

DETD . . . into pRW843 (containing the measles HA gene). Plasmid pRW843

was first digested with NotI and blunt ended with Klenow fragment of **E**. coli DNA polymerase in the presence of 2mM dNTPs. The resulting plasmid, pRW857, therefore contains the measles virus F and. . .

DETD . . . of JEV. First strand cDNA synthesis was primed from a synthetic oligonucleotide complementary to bases 986 to 1005 of the **E** coding region of JEV (FIG. 17A and B) (SEQ ID NO:52). The double-stranded cDNA was ligated to synthetic oligonucleotides containing. . . Biolabs, Beverly, MA), inserted into phosphatase treated EcoRI-cleaved pBR322 (New England Biolabs), and the resulting DNA was used to transform **E**. coli strain DH5 cells (GIBCO/BRL). Plasmids were analyzed by restriction enzyme digestion and a plasmid (pC20) containing cDNA corresponding to 81 nucleotides of non-coding RNA and the C and **prM** coding regions was identified. pC20 was digested at the linker sites with EcoRI and at an internal DraI site situated 28 bp 5' of the ATG initiation codon and the resulting fragment containing the C and **prM** coding regions was inserted into SmaI-EcoRI digested pUC18, creating plasmid, pDr20. The sequence of the C coding region of pC20, combined with an updated sequence of the **prM**, **E**, NS1, NS2A, and NS2B coding regions of the Nakayama strain of JEV is presented in FIG. 17A and B (SEQ. . .

DETD . . . the XhoI and AccI fragment of JEV2 (FIG. 1) containing the plasmid origin and JEV cDNA encoding the carboxy-terminal 40% **prM** and amino-terminal two thirds of **E** (nucleotides 603 to 2124), generating plasmid JEV20 containing JE sequences from the ATG of C through the SacI site (nucleotide 2124) found in the last third of **E**.

DETD . . . 1) in which TTTTTGT nucleotides 1304 to 1310 were changed to TCTTTGT), containing JE sequences from the last third of **E** through the first two amino acids of NS2B (nucleotides 2124 to 4126), the plasmid origin and vaccinia sequences, was ligated. . .

DETD . . . end] generated plasmid JEV25 which contains JE cDNA extending from the SacI site (nucleotide 2124) in the last third of **E** through the carboxy-terminus of **E**. The SacI-EagI fragment from JEV25 was ligated to the SacI-EagI fragment of JEV8 (containing JE cDNA encoding 15 aa C, **prM** and amino-terminal two thirds of **E** nucleotides 337 to 2124, the plasmid origin and vaccinia sequences) yielding plasmid JEV26. A unique SmaI site preceding the ATG. . .

DETD . . . or HpaI-HindIII fragment from JEV7 (FIG. 2) yielded JEV29 [containing a SmaI site followed by JE cDNA encoding 30 aa **E**, NS1, NS2A (nucleotides 2293 to 4125)] and JEV30 [containing a SmaI site followed by JE cDNA encoding 30 aa **E**, NS1, NS2A, NS2B (nucleotides 2293 to 4512)].

DETD HeLa cell monolayers were prepared in 35 mm diameter dishes and infected with vaccinia viruses (**m**.o.i. of 2) or JEV (**m**.o.i. of 5) before radiolabeling. At 16 h post infection, cells were pulse labeled with medium containing ^{35}S-Met and chased. . .

DETD Recombinant vP825 encoded the capsid protein C, structural protein precursor **prM**, the structural glycoprotein **E**, the nonstructural glycoprotein NS1, and the nonstructural protein NS2A (McAda et al., 1987). Recombinant vP829 encoded the putative 15 aa **signal sequence** preceding the amino-terminus of **prM**, as well as **prM**, and **E** (McAda et al., 1987). Recombinant vP857 contained a cDNA encoding the 30 aa hydrophobic carboxy-terminus of **E**, followed by NS1 and NS2A. Recombinant vP864 contained a cDNA encoding the same proteins as vP857 with the addition of NS2B. In recombinants vP825 and vP829 a potential vaccinia virus early transcription termination signal in **E** (TTTTTGT; nucleotides 1399-1405) was modified to TCTTTGT without altering the aa sequence. This change was made in an attempt to increase the level of expression of **E** since this sequence has been shown to increase transcription termination in in vitro transcription assays (Yuen et al., 1987).

DETD **E** and **prM** Were Properly Processed When Expressed by Recombinant Vaccinia Viruses

DETD Pulse-chase experiments demonstrate that proteins identical in size to **E** were synthesized in cells infected with all recombinant vaccinia viruses containing the **E** gene (Table 3). In the case of cells infected with JEV, vP555 and vP829, an **E** protein that migrated slower in SDS-PAGE was also detected in the culture fluid harvested from the

infected cells (Table 3). This extracellular form of **E** produced by JEV- and vP555-infected cells contained mature N-linked glycans (Mason, 1989; Mason et al., 1991), as confirmed for the extracellular forms of **E** produced by vP829-infected cells. Interestingly, vP825, which contained the C coding region in addition to **prM** and **E** specified the synthesis of **E** in a form that is not released into the extracellular fluid (Table 3). Immunoprecipitations prepared from radiolabeled vaccinia-infected cells using a MAb specific for **M** (and **prM**) revealed that **prM** was synthesized in cells infected with vP555, vP825, and vP829, and **M** was detected in the culture fluid of cells infected with vP555 or vP829 (Table 3).

DETD . . . fluids (Table 3). This result indicated that vP829 infected cells produced extracellular particles similar to the empty viral envelopes containing **E** and **M** which are observed in the culture fluids harvested from vP555 infected cells (FIG. 9).

DETD Pre-challenge sera pooled from selected animals in each group were tested for their ability to immunoprecipitate radiolabeled **E** and NS1. The results of these studies (Table 3) demonstrated that: (1) the following order of immune response to **E** vP829>vP555>vP825, (2) all viruses encoding NS1 and NS2A induced antibodies to NS1, and (3) all immune responses were increased by. . . sera collected from these animals (Table 4) confirmed the results of the immunoprecipitation analyses, showing that the immune response to **E** as demonstrated by RIP correlated well with these other serological tests (Table 4).

DETD TABLE 3

Characterization of proteins expressed by vaccinia recombinants and their immune responses

	vP555	vP829	vP825	vP857	vP864
Proteins expressed					
Intracellular	**prM,E** NS1	**prM,E**	**prM,E** NS1	NS1	NS1
secreted	**M,E**,NS1	**M,E**	NS1	NS1	NS1
Particle formation	+	+	-	-	-
Immune response					
single	**E**	**E**	NS1	NS1	NS1
double	**E**,NS1	**E**	**E**,NS1	NS1	NS1

single = single inoculation with 10^7 pfu vaccinia recombinants (ip)
double = two inoculations with 10^7 pfu vaccinia. . .

DETD . . . isolated and ligated to a SacI (JEV nucleotide 2125) to EagI fragment of JEV25 (containing the remaining two thirds of **E**, translation stop and T5NT) generating JEV36. JEV36 was transfected into vP866 (NYVAC) infected cells to generate the vaccinia recombinant vP923. . .

DETD Plasmid YFO containing YF cDNA encoding the carboxy-terminal 80% **prM**, **E** and amino-terminal 80% NS1 (nucleotides 537-3266) was derived by cloning an AvaI to NsiI fragment of YF cDNA (nucleotides 537-1658). . . and KpnI digested IBI25 (International Biotechnologies, Inc., New Haven, CT). Plasmid YF1 containing YF cDNA encoding C and amino-terminal 20% **prM** (nucleotides 119-536) was derived by cloning a RsaI to AvaI fragment of YF cDNA (nucleotides 166-536) and annealed oligos SP46. . . and YF nucleotides 119-165) into AvaI and HindIII digested IBI25. Plasmid YF3 containing YF cDNA encoding the carboxy-terminal 60% of **E** and amino-terminal 25% of NS1 was generated by cloning an ApaI to BamHI fragment of YF CDNA (nucleotides 1604-2725) into. . . cDNA (nucleotides 4339-4940) into SacI and XbaI digested IBI25. Plasmid YF13 containing YF cDNA encoding the carboxy-terminal 25% of C, **prM** and amino-terminal 40% of **E** was derived by cloning a BalI to AnaI fragment of YF cDNA (nucleotides 384-1603) into ApaI and SmaI digested IBI25.

DETD . . . gene in YF1 (TTTTTCT nucleotides 263-269 and TTTTTGT nucleotides 269-275) to (SEQ ID NO:35) TTCTTCTTCTTGT creating plasmid YF1B, in the **E** gene in YF3 (nucleotides 1886-1893 TTTTTTGT to TTCTTTGT

169 aa from the carboxy terminus and nucleotides 2429-2435 TTTTGT to TTCTTGT 8. . . YF3C (nucleotides 1965-2725) was exchanged for the corresponding fragment of YF3B generating YF4 containing YF CDNA encoding the carboxy-terminal 60% **E** and amino-terminal 25% NS1 (nucleotides 1604-2725) with both mutagenized transcription termination signals. An ApaI to BamHI fragment from YF4 (nucleotides 1604-2725) was substituted for the equivalent region in YFO creating plasmid YF6 containing YF cDNA encoding the carboxy-terminal 80% **prM, E** and amino-terminal 80% NS1 (nucleotides 537-3266) with both mutagenized transcription termination signals. Plasmid YF6 was digested with EcoRV within the. . .

DETD . . . mutagenesis described above was used to insert XhoI and ClaI sites preceding the ATG 17 aa from the carboxy-terminus of **E** (nucleotides 2402-2404) in plasmid YF3C creating YF5, to insert XhoI and ClaI sites preceding the ATG 19 aa from the carboxy-terminus of **prM** (nucleotides 917-919) in plasmid YF13 creating YF14, to insert an XhoI site preceding the ATG 23 aa from the carboxy-terminus of **E** (nucleotides 2384-2386) in plasmid YF3C creating plasmid YF25, and to insert an XhoI site and ATG (nucleotide 419) in plasmid. . .

DETD . . . YF5 (nucleotides 1604-2725) was exchanged for the corresponding region of YFO creating YF7 containing YF cDNA encoding the carboxy-terminal 80% **prM, E** and amino-terminal 80% NS1 (nucleotides 537-3266) with XhoI and ClaI sites at 2402 (17 aa from the carboxy-terminus of **E**) and a mutagenized transcription termination signal at 2429-2435 (8 aa from the carboxy-terminus of **E**). The ApaI to BamHI fragment from YF25 (nucleotides 1604-2725) was exchanged for the corresponding region of YFO generating YF26 containing YF cDNA encoding the carboxy-terminal 80% **prM, E** and amino-terminal 80% NS1 (nucleotides 537-3266) with an XhoI site at nucleotide 2384 (23 aa from the carboxy-terminus of **E**) and mutagenized transcription termination signal at 2428-2435 (8 aa from the carboxy-terminus of **E**).

DETD . . . YF14 (nucleotides 537-1603) was substituted for the corresponding region in YF6 generating YF15 containing YF cDNA encoding the carboxy-terminal 80% **prM, E** and amino-terminal 80% NS1 (nucleotides 537-3266) with XhoI and ClaI sites at nucleotide 917 (19 aa from the carboxy-terminus of **prM**) and two mutagenized transcription termination signals. YF6 was digested within IBI25 with EcoRV and within YF at nucleotide 537 with. . .

DETD . . . from YF12 encoding carboxy-terminal 20% NS1, NS2A and NS2B (nucleotides 3267-4569), XhoI to KpnI fragment from YF1S encoding 19 aa **prM, E** and amino-terminal 80% NS1 (nucleotides 917-3266) and XhoI-SmaI digested pHES4 were ligated generating YF23. An XhoI to BamHI fragment from YF26 encoding 23 aa **E**, amino-terminal 25% NS1 (nucleotides 2384-2725) was ligated to an XhoI to BamHI fragment from YF23 (containing the carboxy-terminal 75% NS1,. . .

DETD XhoI-SmaI digested pHES4 was ligated to a purified XhoI to XpnI fragment from YF7 encoding 17 aa **E** and amino-terminal 80% NS1 (nucleotides 2402-3266) plus a KpnI to SmaI fragment from YF10 encoding the carboxy-terminal 20% NS1 and NS2A (nucleotides 3267-4180) creating YF18. An XhoI to BamHI fragment from YF2 encoding C, **prM, E** and amino-terminal 25% NS1 (nucleotides 119-2725) was ligated to a XhoI to BamHI fragment of YF18 (containing the carboxy-terminal 75%. . . the origin of replication and vaccinia sequences) generating YF20. A XhoI to BamHI fragment from YF46 encoding 21 aa C, **prM, E** and amino-terminal 25% NS1 (nucleotides 419-2725) was ligated to the XhoI to BamHI fragment from YF18 generating YF47. Oligonucleotide SP46. . .

DETD Vero cell monolayers were infected with vaccinia virus for 1 hr (**m**.o.i.=10) before radiolabeling. After the absorption period the inoculum was removed and infected cells were overlaid with Met-free media (MEM) containing. . .

DETD HeLa cell monolayers were infected with vaccinia virus (**m**.o.i.=2) or YF17D (**m**.o.i.=4) before radiolabeling. At 38 hr post infection for YF17D or 16 hr post infection for vaccinia, cells were pulsed labeled. . .

DETD Radiolabeled cell lysates and culture fluids were harvested and the viral proteins were immunoprecipitated with monoclonal antibodies to YF

E and NS1 and separated in SDS containing polyacrylamide gels exactly as described by Mason (1989).

DETD Recombinant vP725 encoded the putative 17-aa **signal sequence** preceding the N terminus of the nonstructural protein NS1 and the nonstructural proteins NS1 and NS2A (Rice et al., 1985). Recombinant vP729 encoded the putative 19-aa **signal sequence** preceding the N terminus of **E**, **E**, NS1, NS2A and NS2B (Rice et al., 1985). Recombinant vP764 encoded C, **prM**, **E**, NS1, NS2A and NS2B (Rice et al., 1985). Recombinant vP766 encoded C, **prM**, **E**, NS1 and NS2A (Rice et al., 1985). Recombinant vP869 encoded the putative 21-aa **signal sequence** preceding the N terminus of the structural protein precursor **prM**, **prM E**, NS1 and NS2A (Rice et al., 1985).

DETD **E** Protein Expression By Recombinant Vaccinia Virus

DETD Pulse-chase experiments in HeLa cells demonstrated that a protein identical in size to YF17D **E** was synthesized in cells infected with vP869 and secreted into the culture fluid (Table 7). Under the same conditions of labeling, no intracellular or extracellular **E** was detected in cultures infected with vP766, vP729 or the control vaccinia virus vP457 (Table 7).

DETD Continuous label experiments in Vero cells demonstrated that a protein identical in size to the **E** protein expressed by vP869 was expressed in cultures infected with vP766 and vP729 (Table 7). These results suggest that the **E** protein produced by vP869 infected cells is present in a form in which it is more stable than the **E** protein expressed by vP766 or vP729. YF17D has previously been shown to produce a more labile **E** protein than other YF isolates (Cane et al. 1989).

DETD . . . immunization with YF17D. Pre-challenge sera pooled from selected animals in each group were tested for their ability to immunoprecipitate radiolabeled **E** and NS1 proteins and for the presence of Neut and HAI antibodies. As shown in Table 9 only vP869 and YF17D immunized mice responded to **E** protein, the response was increased by a second inoculation. Mice immunized twice with vP729, vP725 or vP766 produced antibody to. . .

DETD TABLE 7

Characterization of proteins expressed by vaccinia recombinants and YF17D

	17D	vP869	vP729	vP725	vP766	vP457
YF Proteins Expressed Intracellular	**E**,NS1	**E**	**E**,NS1	NS1	**E**,NS1	NONE
Secreted	**E**,NS1	**E**	NS1	NS1	NONE	NONE
Extracellular	YES	YES	NO	NO	NO	NO
HA Activity						

DETD TABLE 9

Pre-challenge Radioimmunoprecipitation

Immunizing Virus	One Inoculation		Two Inoculations	
	Anti-**E**	Anti-NS1	Anti-**E**	Anti-NS1
vP457	-	-	-	-
vP725				+
vP729				+
vP766				+
vP869	+	-	++	-
17D	+	-	++	-

DETD A XhoI to SmaI fragment from YF47 (nucleotides 419-4180) containing YF CDNA encoding 21 amino acids C, **prM**, **E**, NS1, NS2A (with a base missing in NS1 nucleotide 2962) was ligated to XhoI-SmaI digested

SmaI HC (HA region donor plasmid). . . (nucleotide 3262) and a 6700 bp fragment isolated (containing the plasmid origin of replication, vaccinia sequences, 21 amino acids C, **prM**, **E**, amino-terminal 3.5% NS1, carboxy-terminal 23% NS1, NS2A) and ligated to a SacI-Asp718 fragment from YF18 (containing the remainder of NS1. . . site in YF51 were removed using oligonucleotide-directed double-strand break mutagenesis (Mandecki, 1986) creating YF50 encoding YF 21 amino acids C, **prM**, **E**, NS1, NS2A in the HA locus donor plasmid. YF50 was transfected into vP866 (NYVAC) infected cells generating the recombinant vP984. . .

DETD . . . double-strand break mutagenesis creating YF49. Oligonucleotide-directed mutagenesis (Kunkel, 1985) was used to insert a SmaI site at the carboxy-terminus of **E** (nucleotide 2452) in YF4 creating YF16. ApaI-SmaI fragment of YF49 (containing the plasmid origin of replication, vaccinia sequences and YF CDNA encoding 21 amino acids C, **prM**, and amino-terminal 43% **E**) was ligated to an ApaI-SmaI fragment from YF16 (nucleotides 1604-2452 containing the carboxy-terminal 57% **E**) generating YF53 containing 21 amino acids C, **prM**, **E** in the HA locus donor plasmid. YF53 was transfected into vP866 (NYVAC) infected cells generating the recombinant vP1003 (FIG. 19).. . .

DETD Example 13--CLONING OF **DENGUE** TYPE 1 INTO A VACCINIA VIRUS DONOR PLASMID

DETD . . . digested pUC8. An EcoRI-HindIII fragment from DEN1 (nucleotides 2559-3745) and SacI-EcoRI fragment of DEN CDNA encoding the carboxy-terminal 36% of **E** and amino-terminal 16% NS1 (nucleotides 1447-2559, Mason et al., 1987B) were ligated to HindIII-SacI digested IBI24 (International Biotechnologies, Inc., New Haven, Conn.) generating DEN3 encoding the carboxy-terminal 64% **E** through amino-terminal 45% NS2A with a base missing in NS1 (nucleotide 2467).

DETD . . . an AvaI-SacI fragment of DEN cDNA (nucleotides 424-1447 Mason et al., 1987B) generating DEN4 encoding the carboxy-terminal 11 aa C, **prM** and amino-terminal 36% **E**.

DETD Plasmid DEN6 containing DEN cDNA encoding the carboxy-terminal 64% **E** and amino-terminal 18% NS1 (nucleotides 1447-2579 with nucleotide 2467 present Mason et al., 1987B) was derived by cloning a SacI-XhoI. . . Biotechnologies, Inc., New Haven, Conn.). Plasmid DEN15 containing DEN cDNA encoding 51 bases of the DEN 5' untranslated region, C, **prM** and amino-terminal 36% **E** was derived by cloning a HindIII-SacI fragment of DEN cDNA (nucleotides 20-1447, Mason et al., 1987B) into HindIII-SacI digested IBI25.. . .

DETD . . . mutagenesis (Kunkel, 1985) was used to change potential vaccinia virus early transcription termination signals (Yuen et al., 1987) in the **prM** gene in DEN4 29 aa from the carboxy--terminus (nucleotides 822-828 TTTTTCT to TATTTCT) and 13 aa from the carboxy-terminus (nucleotides. . .

DETD . . . (nucleotide 4102) in plasmid DEN23 creating DEN24, to insert a SmaI site and ATG 15 aa from the carboxy-terminus of **E** in DEN7 (nucleotide 2348) creating DEN10, to insert an EagI and HindIII site at the carboxy-terminus of NS2B (nucleotide 4492). . .

DETD . . . DEN7 (nucleotides 1447-2579) was substituted for the corresponding region in DEN3 generating DEN19 containing DEN CDNA encoding the carboxy-terminal 64% **E** and amino-terminal 45% NS2A (nucleotides 1447-3745) with nucleotide 2467 present and the modified transcription termination signal (nucleotides 2448-2454). A XhoI-XbaI. . .

DETD . . . of the H6 promoter and DEN nucleotides 68-494) was ligated to a HindIII-PstI fragment from DEN47 (encoding the carboxy-terminal 83% **prM** and amino-terminal 36% of **E** nucleotides 494-1447 and plasmid origin of replication) generating DEN17 encoding C, **prM** and amino-terminal 36% **E** with part of the H6 promoter and EcoRV site preceding the amino-terminus of C. A HindIII-BlII fragment from DEN17 encoding the carboxy-terminal 13 aa C, **prM** and amino--terminal 36% **E** (nucleotides 370-1447) was ligated to annealed oligonucleotides SP111 and SP112 (containing a disabled HindIII sticky end, EcoRV site to -1. . . a BglII sticky end) creating DEN33 encoding the EcoRV site to -1 of the H6 promoter, carboxy-terminal 20 aa C, **prM** and amino-terminal

DETD 30% **E**.

DETD . . . digested pTP15 (Mason et al., 1991) was ligated to a SmaI-SacI fragment from DEN4 encoding the carboxy-terminal 11 aa C, **prM** and amino-terminal 36% **E** (nucleotides 377-1447) and SacI-EagI fragment from DEN3 encoding the carboxy-terminal 64% **E**, NS1 and amino-terminal 45% NS2A generating DENL. The SacI-XhoI fragment from DEN7 encoding the carboxy-terminal 64% **E** and amino-terminal 18% NS1 (nucleotides 1447-2579) was ligated to a BstEII-SacI fragment from DEN47 (encoding the carboxy-terminal 55% **prM** and amino-terminal 36% **E** (nucleotides 631-1447) and a BstEII-XhoI fragment from DENL (containing the carboxy-terminal 11 aa C, amino-terminal 45% **prM**, carboxy-terminal 82% NS1, carboxy-terminal 45% NS2A, origin of replication and vaccinia sequences) generating DEN8. A unique SmaI site (located between. . .

DETD An EcoRV-SacI fragment from DEN17 (positions -21 to -1 H6 promoter DEN nucleotides 68-1447) encoding C, **prM** and amino-terminal terminal 36% **E**) was ligated to an EcoRV-SacI fragment of DEN8VC (containing vaccinia sequences, H6 promoter from -21 to -124, origin of replication and amino-terminal 64% **E**, NS1, amino-terminal terminal 45% NS2A nucleotides 1447-3745) generating DEN18. A XhoI-EagI fragment from DEN25 encoding the carboxy-terminal 82% NS1 and. . . (nucleotides 2579-4102) was ligated to an XhoI-EagI fragment of DEN18 (containing the origin of replication, vaccinia sequences and DEN C **prM**, **E** and amino-terminal 18% NS1 nucleotides 68-2579) generating DEN26. An EcoRV-SacI fragment from DEN8VC (positions -21 to -1 H6 promoter DEN nucleotides 377-1447 encoding 11aaC, **prM** and amino-terminal 36% **E**) was ligated to an EcoRV-SacI fragment of DEN26 (containing the origin of replication, vaccinia sequences and DEN region encoding the carboxy-terminal 64% **E**, NS1 and NS2A with a base missing in NS1 at nucleotide 2894) generating DEN32. DEN32 was transfected into vP410 infected. . .

DETD . . . DEN10 (nucleotides 1447-2579) was substituted for the corresponding region in DEN3 generating DEN11 containing DEN CDNA encoding the carboxy-terminal 64% **E**, NS1 and amino-terminal 45% NS2A with a SmaI site and ATG 15 aa from the carboxy-terminus of **E**. A SmaI-EagI fragment from DEN11 (encoding the carboxy-terminal 15 aa **E**, NS1 and amino-terminal 45% NS2A nucleotides 2348-3745) was ligated to SmaI-EagI digested pTP15 generating DEN12.

DETD An EcoRV-XhoI fragment from DEN8VC (positions -21 to -1 H6 promoter DEN nucleotides 377-2579 encoding the carboxy-terminal 11 aa C, **prM E**, amino-terminal 18% NS1) was ligated to an EcoRV-XhoI fragment from DEN31 (containing the origin of replication, vaccinia sequences and DEN. . . An EcoRV-SacI fragment from DEN33 (positions -21 to -1 H6 promoter DEN nucleotides 350-1447 encoding the carboxy-terminal 20 aa C, **prM** and amino-terminal 36% **E**) and a SacI-XhoI fragment from DEN32 (encoding the carboxy-terminal 64% **E** and amino-terminal 18% NS1 nucleotides 1447-2579) were ligated to the EcoRV-SacI fragment from DEN31 described above generating DEN34. DEN34 was. . .

DETD . . . vaccinia recombinants vP962, vP955, vP867, vP452 (vaccinia control) or 100 µl of a 10% suspension of suckling mouse brain containing **dengue** type 1 Hawaii strain. Three weeks later sera were collected. One group of mice was re-inoculated and sera were collected. . .

DETD . . . twice with vP962 developed high levels of HAI antibodies, levels were equivalent to those obtained in animals immunized twice with **Dengue** type 1 Hawaii strain.

DETD Construction of Vaccinia Insertion Vector Containing DEN Type 1 20 aaC, **prM, E**

DETD A 338bp fragment encoding the carboxy-terminal 23% **E** (nucleotides 2055-2392, Mason et al., 1987b) TGA stop codon T5NT vaccinia early transcription termination signal (Yuen et al., 1987) and. . . and cloned into HindIII/BamHI digested IBI25 generating DEN36. DEN34 was digested with EcoRV (within the H6 promoter) and HindIII within **E** (DEN nucleotide 2061; Mason et al., 1987b) and a 1733 bp fragment (containing EcoRV to -1 H6 promoter, 20 aaC, **prM** and amino-terminal 77% **E**) was isolated. DEN36 was digested with HindIII and EclXI and a 331 bp fragment isolated (containing DEN nucleotides 2062-2392 TGA. . .

generating plasmid DEN50. Plasmid DEN50 can be transfected into vaccinia infected cells to generate a recombinant encoding DEN 20 aaC, **prM** and **E**.

DETD This example describes the development of canarypox recombinant vCP107 encoding JEV 15aaC, **prM**, **E**, NS1, NS2A and a canarypox donor plasmid (JEVCPC5) encoding 15aaC, **prM**, **E**.

DETD Construction of Insertion Vector Containing JEV 15aaC, **prM**, **E**, NS1, NS2A

DETD Construction of pRW838 is illustrated below (FIG. 23). Oligonucleotides A through **E**, which overlap the translation initiation codon of the H6 promoter with the ATG of rabies G, were cloned into pUC9 as pRW737. Oligonucleotides A through **E** contain the H6 promoter, starting at NruI, through the HindIII site of rabies G followed by BglII. Sequences of oligonucleoties A through **E** are: ##STR16## The diagram of annealed oligonucleotides A through **E** is as follows: ##STR17##

DETD Oligonucleotides A through **E** were kinased, annealed (95° C. for 5 minutes, then cooled to room temperature), and inserted between the PvuII sites of. . .

DETD . . . promoter, plasmid origin of replication and C5 flanking arms isolated. Plasmid JEVL14VC containing JEV cDNA encoding 15 amino acids C, **prM**, **E**, NS1, NS2A in a vaccinia virus donor plasmid (FIG. 1) (nucleotides 337-4125, FIG. 17A and B) (SEQ ID NO:52) was. . . JEVCP1 was transfected into ALVAC infected primary CEF cells to generate the canarypox recombinant vCP107 encoding 15 amino acids C, **prM**, **E**, NS1, NS2A (FIG. 18).

DETD Construction of C5 Insertion Vector Containing JEV 15aac, **prM**, **E**

DETD . . . annealed oligonucleotides SP131 (SEQ ID NO:75) and SP132 (SEQ ID NO:76) (containing a SphI sticky end, T nucleotide completing the **E** coding region, translation stop, a vaccinia early transcription termination signal (AT5AT; Yuen and Moss, 1987), a second translation stop, and XbaI sticky end) generating plasmid JEVCP5 which encodes 15 amino acids C, **prM** and **E** under the control of the H6 promoter between C5 flanking arms. JEVCP5 can be transfected in ALVAC or ALVAC recombinant infected cells to generate a recombinant encoding JEV 15 aa C, **prM** and **E**. ##STR19## Example 15--CONSTRUCTION OF ALVAC RECOMBINANT EXPRESSING YFV PROTEINS Construction of Canarypox Insertion Vector

DETD . . . and SmaI and ligated to a 3772 bp XhoI-SmaI fragment from YF51 (nucleotides 419-4180 encoding YF 21 amino acids C, **prM**, **E**, NS1, NS2A) generating YF52. The 6 bp corresponding to the unique XhoI site in UP52 were removed using oligonucleotide-directed double-strand. . . YFCP3. YFCP3 was transfected into ALVAC infected primary CEF cells to generate the canarypox recombinant vCPI27 encoding 21 aa C, **prM**, **E**, NS1, NS2A (FIG. 19).

DETD Construction of C3 Insertion Vector Containing YFV 21 aa C, **prM**, **E**

DETD . . . 8344 bp fragment isolated (containing the plasmid origin of replication, canarypox DNA and YF cDNA encoding 21 amino acids C, **prM**, and amino-terminal 57% **E**) and ligated to a ApaI to SmaI fragment from YF16 (nucleotides 1604-2452 containing the carboxy-terminal 43% **E**) generating YF54. The 6 bp corresponding to the unique XhoI site in YF54 were removed as described above creating YFCP4 containing YF cDNA encoding 21 amino acids C, **prM**, and **E**. YFCP4 can be transfected into ALVAC or ALVAC recombinant infected cells to generate a recombinant encoding YFV 21 aa C, **prM**, **E**.

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1. A recombinant poxvirus comprising DNA coding for at least one **flavivirus** structural protein, wherein the **flavivirus** is **Yellow Fever virus** or **Dengue** virus and the poxvirus is selected from the group consisting of: an avipox virus, a vaccinia virus wherein the open. . .

2. The recombinant poxvirus of claim 1 wherein the DNA comprises a part of the **flavivirus** open reading frame from c to NS2b.

3. The recombinant poxvirus of claim 1 wherein the DNA encodes protein **M** or a precursor to protein **M**; and **flavivirus** proteins **E**, NS1 and NS2A.

7. The recombinant poxvirus of claim 1 wherein the **flavivirus** is **Yellow Fever virus**.

8. The recombinant poxvirus of claim 1 wherein the **flavivirus** is **Dengue** virus.

17. A method for producing a **flavivirus** structural protein comprising introducing into a cell a recombinant poxvirus, transforming cell with the expression vector, cultivating the transformed cell. . .

L14 ANSWER 13 OF 15 USPATFULL on STN

1998:44885 **Flavivirus** recombinant poxvirus vaccine.

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DOCUMENT TYPE: Utility; Granted.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB What is described is a recombinant poxvirus, such as vaccinia virus, fowlpox virus and canarypox virus, containing foreign DNA from **flavivirus**, such as Japanese **encephalitis virus, yellow fever virus** and **Dengue** virus. In a preferred embodiment, the recombinant poxvirus generates an extracellular particle containing **flavivirus E** and **M** proteins capable of inducing neutralizing antibodies, hemagglutination-inhibiting antibodies and protective immunity against

flavivirus infection. What is also described is a vaccine containing the recombinant poxvirus for inducing an immunological response in a host animal inoculated with the vaccine.

CLM What is claimed is:

1. A recombinant avipox virus comprising DNA coding for Japanese **encephalitis virus** protein **M** or a precursor to protein **M**, and Japanese **encephalitis virus** protein **E**, NS1 and NS2A, in a nonessential region of the avipox genome.

2. A recombinant avipox virus as in claim 1 wherein the avipox virus is canarypox virus.

3. A recombinant avipox virus as in claim 2 wherein the canarypox virus is ALVAC or a canarypox virus attenuated through more than 200 serial passages on chick embryo fibroblasts, subjected to four successive plaque purifications, and amplified through five additional serial passages.

4. A recombinant avipox virus comprising DNA from Japanese **encephalitis virus** (JEV) in a nonessential region of the avipox genome, wherein the DNA comprises the part of the JEV open reading frame extending from **prM** to NS2a.

5. A recombinant avipox virus as in claim 4, wherein the part of the JEV open reading frame further comprises the DNA encoding 15 C-terminal amino acids of C.

6. A recombinant avipox virus as in claim 4, wherein the part of the JEV open reading frame further comprises NS2b.

7. The recombinant avipox virus of claim 4 which is a canarypox virus which is ALVAC or a canarypox virus attenuated through more than 200 serial passages on chick embryo fibroblasts, subjected to four successive plaque purifications, and amplified through five additional serial passages.

8. An immunological composition comprising a carrier and an avipox virus according to claim 1, wherein the composition is effective to induce an immunological response in a host animal.

9. An immunological composition comprising a carrier and an avipox virus according to claim 3, wherein the composition is effective to induce an immunological response in a host animal.

10. An immunological composition comprising a carrier and an avipox virus according to claim 4, wherein the composition is effective to induce an immunological response in a host animal.

11. An immunological composition comprising a carrier and an avipox virus according to claim 7, wherein the composition is effective to induce an immunological response in a host animal.

12. A vaccine composition comprising a carrier and an avipox virus according to claim 1, wherein the composition is effective to induce a protective response against Japanese **encephalitis virus** in a host animal.

13. A vaccine composition comprising a carrier and an avipox virus according to claim 3, wherein the composition is effective to induce a protective response against Japanese **encephalitis virus** in a host animal.

14. A vaccine composition comprising a carrier and an avipox virus according to claim 4, wherein the composition is effective to induce a protective response against Japanese **encephalitis virus** in a host animal.

15. A vaccine composition comprising a carrier and an avipox virus according to claim 7, wherein the composition is effective to induce a protective response against Japanese **encephalitis virus** in a host animal.

16. The recombinant avipox virus of claim 4 which is vcP107.

TI **Flavivirus** recombinant poxvirus vaccine

AI US 1994-224391 19940407 (8) <--

AB What is described is a recombinant poxvirus, such as vaccinia virus, fowlpox virus and canarypox virus, containing foreign DNA from **flavivirus**, such as Japanese **encephalitis virus**, **yellow fever virus** and **Dengue** virus. In a preferred embodiment, the recombinant poxvirus generates an extracellular particle containing **flavivirus E** and **M** proteins capable of inducing neutralizing antibodies, hemagglutination-inhibiting antibodies and protective immunity against **flavivirus** infection. What is also described is a vaccine containing the recombinant poxvirus for inducing an immunological response in a host. . .

SUMM . . . and using the same. More in particular, the invention relates to recombinant poxvirus, which virus expresses gene products of a **flavivirus** gene, and to vaccines which provide protective immunity against **flavivirus** infections.

SUMM . . . sequence to be inserted into the virus, particularly an open reading frame from a non-pox source, is placed into an **E**. coli plasmid construct into which DNA homologous to a section of DNA of the poxvirus has been inserted. Separately, the. . . flanking a region of pox DNA containing a nonessential locus. The resulting plasmid construct is then amplified by growth within **E**. coli bacteria (Clewell, 1972) and isolated (Clewell et al., 1969; Maniatis et al., 1986).

SUMM Second, the isolated plasmid containing the DNA gene sequence to be inserted is transfected into a cell culture, **e**.g. chick embryo fibroblasts, along with the poxvirus. Recombination between homologous pox DNA in the plasmid and the viral genome respectively. . .

SUMM The family **Flaviviridae** comprises approximately 60 arthropod-borne viruses that cause significant public health problems in both temperate and tropical regions of the world. . . developed against some of these agents, there has been a recent surge in the study of the molecular biology of **flaviviruses** in order to produce recombinant vaccines to the remaining viruses, most notably **dengue** (Brandt, 1988).

SUMM **Flavivirus** proteins are encoded by a single long translational open reading frame (ORF) present in the positive-strand genomic RNA. The genes. . . end of the genome followed by the nonstructural glycoprotein NS1 and the remaining nonstructural proteins (Rice et al., 1985). The **flavivirus** virion contains an **envelope** glycoprotein, **E**, a **membrane** protein, **M**, and a capsid protein, C. In the case of Japanese **encephalitis virus** (JEV), virion preparations usually contain a small amount of the glycoprotein precursor to the **membrane** protein, **prM** (Mason et al., 1987a). Within JEV-infected cells, on the other hand, the **M** protein is present almost exclusively as the higher molecular weight **prM** protein (Mason et al., 1987a; Shapiro et al., 1972).

SUMM Studies that have examined the protective effect of passively administered monoclonal antibodies (MAbs) specific for each of the three **flavivirus** glycoproteins (**prM**, **E**, NS1) have demonstrated that immunity to each of these antigens results in partial or complete protection from lethal viral challenge. Monoclonal antibodies to **E** can provide protection from infection by Japanese **encephalitis virus** (JEV) (Kimura-Kuroda et al., 1988; Mason et al., 1989), **dengue** type 2 virus (Kaufman et al., 1987) and **yellow fever virus** (YF) (Gould et al., 1986). In most cases, passive protection has been correlated with the ability of these **E** MAbs to neutralize the virus in vitro. Recently, Kaufman et al. (1989) have demonstrated that passive protection can also be produced with **prM** MAbs that exhibit weak or undetectable neutralizing activity in vitro. The ability of structural

protein specific MAbs to protect animals. . . attenuate viral infection by blocking virus binding to target cells. Passive protection experiments using MAbs to the NS1 protein of **yellow fever virus** (Schlesinger et al., 1985; Gould et al., 1986) and **dengue** type 2 virus (Henchal et al., 1988) have demonstrated that antibodies to this nonstructural glycoprotein can protect animals from lethal. . .

SUMM . . . of NS1 immunity to protect the host from infection comes from direct immunization experiments in which NS1 purified from either **yellow fever virus**-infected cells (Schlesinger et al., 1985, 1986) or **dengue** type 2 virus-infected cells (Schlesinger et al., 1987) induced protective immunity from infection with the homologous virus.

SUMM Although significant progress has been made in deriving the primary structure of these three **flavivirus** glycoprotein antigens, less is known about their three-dimensional structure. The ability to produce properly folded, and possibly correctly assembled, forms. . . NS1-based vaccines, dimerization of NS1 (Winkler et al., 1988) may be required to elicit the maximum protective response. For the **E** protein, correct folding is probably required for eliciting a protective immune response since **E** protein antigens produced in **E**. coli (Mason et al., 1989) and the authentic **E** protein prepared under denaturing conditions (Wengler et al., 1989b) failed to induce neutralizing antibodies. Correct folding of the **E** protein may require the coordinated synthesis of the **prM** protein, since these proteins are found in heterodimers in the cell-associated forms of West Nile virus (Wengler et al., 1989a). The proper folding of **E** and the assembly of **E** and **prM** into viral particles may require the coordinated synthesis of the NS1 protein, which is coretained in an early compartment of the secretory apparatus along with immature forms of **E** in JEV-infected cells (Mason, 1989).

SUMM Attempts to produce recombinant **flavivirus** vaccines based on the **flavivirus** glycoproteins has met with some success, although protection in animal model systems has not always correlated with the predicted production. . .

SUMM . . . a vaccinia recombinant containing the region of JEV encoding 65 out of the 127 amino acids of C, all of **prM**, all of **E**, and 59 out of the 352 amino acids of NS1. Haishi et al. (1989) reported a vaccinia recombinant containing Japanese encephalitis sequences encoding 17 out of the 167 amino acids of **prM**, all of **E** and 57 out of the 352 amino acids of NS1.

SUMM Deubel et al. (1988) reported a vaccinia recombinant containing the **dengue**-2 coding sequences for all of C, all of **prM**, all of **E** and 16 out of the 352 amino acids of NS1.

SUMM Zhao et al. (1987) reported a vaccinia recombinant containing the **dengue**-4 coding sequences for all of C, all of **prM**, all of **E**, all of NS1, and all of NS2A. Bray et al. (1989) reported a series of vaccinia recombinants containing the **dengue**-4 coding sequences for (i) all of C, all of **prM** and 416 out of the 454 amino acids of **E**, (ii) 15 out of the 167 amino acids of **prM** and 416 out of the 454 amino acids of **E**, (iii) 18 amino acids of influenza A virus hemagglutinin and 416 out of the 454 amino acids of **E**, and (iv) 71 amino acids of respiratory syncytial virus G glycoprotein and 416 out of the 454 amino acids of **E**.

SUMM Despite these attempts to produce recombinant **flavivirus** vaccines, the proper expression of the JEV **E** protein by the vaccinia recombinants has not been satisfactorily obtained. Although Haishi et al. (1989) demonstrated cytoplasmic expression of JEV **E** protein by their vaccinia recombinant, the distribution was different from that observed in JEV infected cells. Yasuda et al. (1990) detected expression of JEV **E** protein by their vaccinia recombinant on the cell surface. Recombinant viruses that express the **prM** and **E** protein protected mice from approximately 10 LD_{50} of challenge virus. Yasuda et al. (1990) elicited anti-JEV immune responses as well as protection but reactivity to a panel of **E** specific monoclonal antibodies exhibited differences from the reactivity observed in JEV infected cells.

SUMM **Dengue** type 2 structural proteins have been expressed by recombinant vaccinia viruses (Deubel et al., 1988). Although these viruses induced the synthesis of the structural glycoprotein within infected cells, they

neither elicited detectable anti **dengue** immune responses nor protected monkeys from **dengue** infection. Several studies also have been completed on the expression of portions of the **dengue** type 4 structural and nonstructural proteins in vaccinia virus (Bray et al., 1989; Falgout et al., 1989; Zhao et al.,. . . . the viral ORF extending from C to NS2A under the control of the P7.5 early-late promoter produced intracellular forms of **prM**, **E**, and NS1 but failed to induce the synthesis of extracellular forms of any of the structural proteins, even though a. . . . this recombinant virus (Bray et al., 1989; Zhao et al., 1987). Additional recombinant viruses that contained several forms of the **dengue** type 4 **E** gene with or without other structural protein genes have also been examined (Bray et al., 1989). Although several of these recombinant viruses were able to induce protection, they neither produced extracellular forms of **E** nor induced neutralizing antibodies. A **dengue**-vaccinia recombinant expressing a C-terminally truncated **E** protein gene induced the synthesis of an extracellular form of **E** and provided an increasing level of resistance to **dengue** virus encephalitis in inoculated mice (Men et al., 1991).

SUMM It can thus be appreciated that provision of a **flavivirus** recombinant poxvirus which produces properly processed forms of **flavivirus** proteins, and of vaccines which provide protective immunity against **flavivirus** infections, would be a highly desirable advance over the current state of technology.

SUMM It is therefore an object of this invention to provide recombinant poxviruses, which viruses express properly processed gene products of **flavivirus**, and to provide a method of making such recombinant poxviruses.

SUMM It is an additional object of this invention to provide for the cloning and expression of **flavivirus** coding sequences in a poxvirus vector.

SUMM It is another object of this invention to provide a vaccine which is capable of eliciting **flavivirus** neutralizing antibodies, hemagglutination-inhibiting antibodies and protective immunity against **flavivirus** infection and a lethal **flavivirus** challenge.

SUMM In one aspect, the present invention relates to a recombinant poxvirus generating an extracellular **flavivirus** structural protein capable of inducing protective immunity against **flavivirus** infection. In particular, the recombinant poxvirus generates an extracellular particle containing **flavivirus** **E** and **M** proteins capable of eliciting neutralizing antibodies and hemagglutination-inhibiting antibodies. The poxvirus is advantageously a vaccinia virus or an avipox virus, such as fowlpox virus or canarypox virus. The **flavivirus** is advantageously Japanese **encephalitis virus**, **yellow fever virus** and **Dengue** virus.

SUMM According to the present invention, the recombinant poxvirus contains therein DNA from **flavivirus** in a nonessential region of the poxvirus genome for expressing in a host **flavivirus** structural protein capable of release to an extracellular medium. In particular, the DNA contains Japanese **encephalitis virus** coding sequences that encode a precursor to structural protein **M**, structural protein **E**, and nonstructural proteins NS1 and NS2A. More in particular, the recombinant poxvirus contains therein DNA from **flavivirus** in a nonessential region of the poxvirus genome for expressing a particle containing **flavivirus** structural protein **E** and structural protein **M**.

SUMM . . . with the vaccine, said vaccine including a carrier and a recombinant poxvirus containing, in a nonessential region thereof, DNA from **flavivirus**.

SUMM More in particular, the recombinant viruses express portions of the **flavivirus** ORF extending from **prM** to NS2B. Biochemical analysis of cells infected with the recombinant viruses showed that the recombinant viruses specify the production of properly processed forms of all three **flavivirus** glycoproteins--**prM**, **E**, and NS1. The recombinant viruses induced synthesis of extracellular particles that contained fully processed forms of the **M** and **E** proteins. Furthermore, the results of mouse immunization studies demonstrated that the induction of neutralizing antibodies and high levels of protection were associated with the ability of the immunizing recombinant viruses to produce extracellular particles containing the two structural **membrane** proteins.

DRWD FIG. 7 shows a comparison by SDS-PAGE analysis of the cell lysate **E** proteins produced by JEV infection and infection with the recombinant vaccinia viruses vP650, vP555, vP658 and vP583;

DRWD FIG. 8 shows a comparison by SDS-PAGE analysis of the culture fluid **E** proteins produced by JEV infection and infection with the recombinant vaccinia viruses vP650, vP555, vP658 and vP583;

DRWD FIG. 9 shows a comparison by sucrose gradient analysis of the forms of the **E** protein found in the culture fluid harvested from JEV infected cells and cells infected with vaccinia recombinants vP555 and vP650;

DETD . . . and B (SEQ ID NO:52) which contains the sequence of the C coding region combined with an updated sequence of **prM**, **E**, NS1, NS2A and NS2B coding regions.

DETD . . . The resulting plasmid, pJEV1, contained the viral ORF extending from the SacI site (nucleotide 2125) in the last third of **E** through the BalI site (nucleotide 4125) two amino acid residues (aa) into the predicted N terminus of NS2B (FIG. 1).

DETD . . . containing a XhoI sticky end, a SmaI site, the last 15 aa of C, and first 9 aa of JEV **prM** with a sticky HindIII end) were ligated to a HindIII-SacI fragment of JEV cDNA (nucleotides 407-2124), and XhoI-SacI digested vector. . . the viral ORF extending between the methionine (Met) codon (nucleotides 337-339) occurring 15 aa preceding the predicted N terminus of **prM** and the SacI site (nucleotide 2124) found in the last third of **E** (FIG. 1).

DETD . . . pJEV5, contained the viral ORF extending between the Met codon (nucleotides 811-813) occurring 25 aa preceding the N terminus of **E** and the SacI site (nucleotide 2124) found in the last third of **E** (FIG. 1).

DETD . . . (Kunkel, 1985) was used to change a potential vaccinia virus early transcription termination signal (Yuen et al., 1987) in the **E** gene of pJEV2 (TTTTTGT; nucleotides 1304-1310) to TCTTTGT, creating plasmid pJEV22 (FIG. 2). The same change was performed on pJEV5. . .

DETD . . . resulting plasmid, pJEV7, contained the viral ORF extending between the SacI site (nucleotide 2125) found in the last third of **E** and the last codon of NS2B (nucleotide 4512) (FIG. 2). SmaI-EagI digested pTP15 was purified and ligated to the purified. . .

DETD BHK or VERO cell monolayers were prepared in 35 mm diameter dishes and infected with vaccinia viruses (**m**.o.i. of 2) or JEV (**m**.o.i. of 5) and incubated for 11 hr (vaccinia) or 16 hr (JEV) before radiolabeling. At 11 hr or 16 hr. . .

DETD Four different vaccinia virus recombinants were constructed that expressed portions of the JEV coding region extending from **prM** through NS2B. The JEV cDNA sequences contained in these recombinant viruses are shown in FIG. 4. In all four recombinant. . .

DETD Recombinant vP555 encodes the putative 15 aa **signal sequence** preceding the N terminus of the structural protein precursor **prM**, the structural glycoprotein **E**, the nonstructural glycoprotein NS1, and the nonstructural protein NS2A (McAda et al., 1987). Recombinant vP583 encodes the putative **signal sequence** preceding the N terminus of **E**, **E**, NS1, and NS2A (McAda et al., 1987). Recombinant vP650 contains a cDNA encoding the same proteins as vP555 with the. . . vP583 with the addition of NS2B. In recombinants vP650 and vP658, a potential vaccinia virus early transcription termination signal in **E** (TTTTTGT; nucleotides 1087-1094) was modified to TCTTTGT without altering the aa sequence. This change was made in an attempt to increase the level of expression of **E** and NS1, since this sequence has been shown to increase transcription termination in in vitro transcription assays (Yuen et al.,. . .

DETD . . . noted that recombinants vP555, vP583, and vP650 had a deletion from within the HindIII C fragment through HindIII N and **M** and into HindIII K. This same deletion was observed in the vP425 parental virus. Interestingly, these viruses were less cytopathic. . .

DETD . . . lysate (FIG. 5) or culture fluid (FIG. 6) prepared from each cell layer were immunoprecipitated, and then either mock digested (**M**), digested with endo H (H), or digested with PNGase F (F), prior to SDS-PAGE analysis.

DETD . . . production by all four recombinants, suggesting that the

potential vaccinia early transcriptional termination signal present near the end of the **E** coding region in vP555 and vP583 did not significantly reduce the amount of NS1 produced relative to vP650 or vP658. . .

DETD **E** and **prM** were Properly Processed when Expressed by Recombinant Vaccinia Viruses

DETD FIGS. 7 and 8 show a comparison of the **E** protein produced by JEV infection or infection with the recombinant vaccinia viruses. BHK cells were infected with JEV or recombinant. . . . lysate (FIG. 7) or culture fluid (FIG. 8) prepared from each cell layer were immunoprecipitated, and then either mock digested (**M**), digested with endo H (H), or digested with PNGase F (F), prior to SDS-PAGE analysis.

DETD The data from the pulse-chase experiments depicted in FIGS. 7 and 8 demonstrate that proteins identical in size to **E** were synthesized in cells infected with all recombinant vaccinia viruses containing the **E** gene. However, the **E** protein was only released from cells infected with vaccinia viruses that contained the region of the viral ORF encoding **prM**, **E**, NS1, and NS2A (vP555 and vP650; see FIGS. 4, 7 and 8). Endoglycosidase sensitivity (FIGS. 7 and 8) revealed that both the intracellular and extracellular forms of the **E** protein synthesized by cells infected with the vaccinia recombinants were glycosylated; the cell-associated forms of **E** were endo H sensitive, whereas the extracellular forms were resistant to endo H digestion.

DETD Immunoprecipitates prepared from radiolabeled vaccinia-infected cells using a MAb specific for **M** (and **prM**) revealed that **prM** was synthesized in cells infected with vP555 and vP650. Cells infected with either of these recombinant vaccinia viruses produced cellular forms of **prM** that were identical in size to the **prM** protein produced by JEV-infected cells, and a **M** protein of the correct size was detected in the culture fluid of cells infected with these two viruses.

DETD The extracellular fluid harvested from cells infected with vP555 and vP650 contained forms of **E** that migrated with a peak of hemagglutinating activity in sucrose density gradients. Interestingly, this hemagglutinin migrated similarly to the slowly. . . found in the culture fluid of JEV-infected cells (FIG. 9). Furthermore, these same fractions contained the fully processed form of **M**, demonstrating that vP555- and vP650-infected cells produced a particle that contained both of the structural **membrane** proteins of JEV. These particles probably represent empty JEV envelopes, analogous to the 22 nm hepatitis B virus particles found. . .

DETD Recombinant vaccinia virus vP555 produced **E**- and **M**-containing extracellular particles that behaved like empty viral envelopes. The ability of this recombinant virus to induce the synthesis of extracellular. . .

DETD The recombinant viruses described herein contain portions of the JEV ORF that encode the precursor to the structural protein **M**, the structural protein **E**, and nonstructural proteins NS1, NS2A, and NS2B. The **E** and NS1 proteins produced by cells infected with these recombinant viruses underwent proteolytic cleavage and N-linked carbohydrate addition in a. . . proteins produced by cells infected with JEV. These data further demonstrate that the proteolytic cleavage and N-linked carbohydrate addition to **E** and NS1 do not require **flavivirus** nonstructural proteins located 3' to NS2A in the viral genome (Bray et al., 1989; Deubel et al., 1988; Falgout et. . .

DETD . . . the portion of the ORF inserted in the recombinant vaccinia viruses had a significant effect on the late-stage processing of **prM** and **E**, but not on the fate of NS1. All recombinant viruses that encoded NS1 produced mature extracellular forms of this protein,. . . from transfected cells (Fan et al., 1990). On the other hand, only two of the four recombinants that contained the **E** protein coding region produced extracellular forms of **E**. These two recombinants, vP555 and vP650, differed from the remaining recombinants in that they contained the **prM** coding region in addition to **E**, NS1, and NS2A. The findings that extracellular forms of **E** were produced only by viruses containing the coding regions for both **E** and **prM** and that the extracellular forms of **E** were associated with **M** suggest that the simultaneous

synthesis of **prM** and **E** is a requirement for the formation of particles that are targeted for the extracellular fluid.

DETD . . . the co-migration of the rabbit immunoglobulin heavy chain with the radiolabeled viral antigens, and to permit clear separation of the **E** and the NS1' proteins. Neutralization tests were performed on heat-inactivated sera (20 min. at 56° C.) as described (Tesh et. . .

DETD . . . virus, two viruses (vP555 and vP658) were selected for in-depth challenge studies. vP555 induced the synthesis of extracellular forms of **E**, whereas vP658 did not produce any extracellular forms of **E**, but contained additional cDNA sequences encoding the NS2B protein. In the challenge experiments several dilutions of challenge virus were tested,. . . dose of JEV. The analysis demonstrated that: (1) only those animals immunized with vP555 showed a strong immune response to **E**, and (2) a second inoculation resulted in a significant increase in reactivity to the **E** protein (FIG. 10).

DETD . . . induce neutralizing antibodies may be related to the fact that vP555 produces an extracellular particulate form of the structural proteins **E** and **M**. This SHA-like particle probably represents an empty JEV **envelope** that contains **E** and **M** folded and assembled into a configuration very similar to that found in the infectious JEV particle. Recombinants vP555 and vP650. . . assembly of viral envelopes. Other investigators (see above) have not been able to detect the production of extracellular forms of **E** by cells expressing all three structural proteins (C, **prM**, and **E**) in the presence or absence of NS1 and NS2A. The inability of their recombinant viruses to produce particles similar to. . . that the C protein produced in the absence of a genomic RNA interferes with the proper assembly of the viral **membrane** proteins. Alternatively, an incompletely processed form of C similar to that detected by Nowak et al. (1989) in in vitro translation experiments, could prevent release of the structural **membrane** proteins from the cells expressing the C gene.

DETD . . . were obtained from GIBCO/BRL, Gaithersburg, Md., New England Biolabs, Beverly, Mass.; and Boehringer Mannheim Biochemicals, Indianapolis, Ind. Klenow fragment of **E**. coli polymerase was obtained from Boehringer Mannheim Biochemicals. BAL-31 exonuclease and phage T4 DNA ligase were obtained from New England. . .

DETD . . . NcoI site (pos. 172,253) were removed by digestion of pSD419 with NcoI/SmaI followed by blunt ending with Klenow fragment of **E**. coli polymerase and ligation generating plasmid pSD476. A vaccinia right flanking arm was obtained by digestion of pSD422 with HpaI. . . ID NO:6/SEQ ID NO:7) ##STR3## generating pSD479. pSD479 contains an initiation codon (underlined) followed by a BamHI site. To place **E**. coli Beta-galactosidase in the B13-B14 (u) deletion locus under the control of the u promoter, a 3.2 kb BamHI fragment. . .

DETD . . . at the pUC/vaccinia junction was destroyed by digestion of pSD478 with EcoRI followed by blunt ending with Klenow fragment of **E**. coli polymerase and ligation, generating plasmid pSD478E$^-$. pSD478E$^-$ was digested with BamHI and HpaI and ligated with annealed synthetic oligonucleotides. . .

DETD . . . XbaI within vaccinia sequences (pos. 137,079) and with HindIII at the pUC/vaccinia junction, then blunt ended with Klenow fragment of **E**. coli polymerase and ligated, resulting in plasmid pSD483. To remove unwanted vaccinia DNA sequences to the right of the A26L. . . digestion with BglII (pos. 140,136) and with EcoRI at the pUC/vaccinia junction, followed by blunt ending with Klenow fragment of **E**. coli polymerase and ligation. The resulting plasmid was designated pSD489. The 1.8 kb ClaI (pos. 137,198)/EcoRV (pos. 139,048) fragment from. . .

DETD A 3.3 kb BglII cassette containing the **E**. coli Beta-galactosidase gene (Shapira et al., 1983) under the control of the vaccinia 11 kDa promoter (Bertholet et al., 1985;. . .

DETD A 3.2 kb BglII/BamHI (partial) cassette containing the **E**. coli Beta-galactosidase gene (Shapira et al., 1983) under the control of the vaccinia 11 kDa promoter (Bertholet et al., 1985;. . .

DETD . . . were removed from the pUC/vaccinia junction by digestion of pSD466 with EcoRI/BamHI followed by blunt ending with Klenow fragment of

E. coli polymerase and ligation. Recombination between vP708 and pSD467 resulted in recombinant vaccinia deletion mutant, vP723, which was isolated as. . .

DETD . . . SphI and religated, forming pSD451. In pSD451, DNA sequences to the left of the SphI site (pos. 27,416) in HindIII **M** are removed (Perkus et al., 1990). pSD409 is HindIII **M** cloned into pUC8.

DETD To provide a substrate for the deletion of the [C7L-K1L] gene cluster from vaccinia, **E**. coli Beta-galactosidase was first inserted into the vaccinia M2L deletion locus (Guo et al., 1990) as follows. To eliminate the. . . unique BglII site inserted into the M2L deletion locus as indicated above. A 3.2 kb BamHI (partial)/BglII cassette containing the **E**. coli Beta-galactosidase gene (Shapira et al., 1983) under the control of the 11 kDa promoter (Bertholet et al., 1985) was. . .

DETD . . . deleted for vaccinia genes [C7L-K1L] was assembled in pUC8 cut with SmaI, HindIII and blunt ended with Klenow fragment of **E**. coli polymerase. The left flanking arm consisting of vaccinia HindIII C sequences was obtained by digestion of pSD420 with XbaI (pos. 18,628) followed by blunt ending with Klenow fragment of **E**. coli polymerase and digestion with BglII (pos. 19,706). The right flanking arm consisting of vaccinia HindIII K sequences was obtained. . .

DETD . . . coding sequences, pSD518 was digested with BamHI (pos. 65,381) and HpaI (pos. 67,001) and blunt ended using Klenow fragment of **E**. coli polymerase. This 4.8 kb vector fragment was ligated with a 3.2 kb SmaI cassette containing the **E**. coli Beta-galactosidase gene (Shapira et al., 1983) under the control of the vaccinia 11 kDa promoter (Bertholet et al., 1985;. . .

DETD . . . mutagenized expression cassette contained within pRW837 was derived by digestion with HindIII and EcoRI, blunt-ended using the Klenow fragment of **E**. coli DNA polymerase in the presence of 2 mM dNTPs, and inserted into the SmaI site of pSD513 to yield. . .

DETD . . . into pRW843 (containing the measles HA gene). Plasmid pRW843 was first digested with NotI and blunt-ended with Klenow fragment of **E**. coli DNA polymerase in the presence of 2 mM dNTPs. The resulting plasmid, pRW857, therefore contains the measles virus F. . .

DETD . . . of JEV. First strand cDNA synthesis was primed from a synthetic oligonucleotide complementary to bases 986 to 1005 of the **E** coding region of JEV (FIGS. 17A and B) (SEQ ID NO:52). The double-stranded cDNA was ligated to synthetic oligonucleotides containing. . . Biolabs, Beverly, Mass.), inserted into phosphatase treated EcoRI-cleaved pBR322 (New England Biolabs), and the resulting DNA was used to transform **E**. coli strain DH5 cells (GIBCO/BRL). Plasmids were analyzed by restriction enzyme digestion and a plasmid (pC20) containing cDNA corresponding to 81 nucleotides of non-coding RNA and the C and **prM** coding regions was identified. pC20 was digested at the linker sites with EcoRI and at an internal DraI site situated 28 bp 5' of the ATG initiation codon and the resulting fragment containing the C and **prM** coding regions was inserted into SmaI-EcoRI digested pUC18, creating plasmid, pDr20. The sequence of the C coding region of pC20, combined with an updated sequence of the **prM**, **E**, NS1, NS2A, and NS2B coding regions of the Nakayama strain of JEV is presented in FIGS. 17A and B (SEQ. . .

DETD . . . the XhoI and AccI fragment of JEV2 (FIG. 1) containing the plasmid origin and JEV cDNA encoding the carboxy-terminal 40% **prM** and amino-terminal two thirds of **E** (nucleotides 603 to 2124), generating plasmid JEV20 containing JE sequences from the ATG of C through the SacI site (nucleotide 2124) found in the last third of **E**.

DETD . . . 1) in which TTTTTGT nucleotides 1304 to 1310 were changed to TCTTTGT), containing JE sequences from the last third of **E** through the first two amino acids of NS2B (nucleotides 2124 to 4126), the plasmid origin and vaccinia sequences, was ligated. . .

DETD . . . end] generated plasmid JEV25 which contains JE cDNA extending from the SacI site (nucleotide 2124) in the last third of **E** through the carboxy-terminus of **E**. The SacI-EaQI fragment from JEV25 was ligated to the SacI-EagI fragment of JEV8 (containing JE cDNA encoding 15 aa C, **prM** and amino-terminal two thirds of **E** nucleotides 337 to 2124, the plasmid origin and vaccinia sequences) yielding plasmid JEV26. A unique SmaI site preceding the ATG. . .

DETD . . . of HpaI-HindIII fragment from JEV (FIG. 2) yielded JEV29 [containing a SmaI site followed by JE cDNA encoding 30 aa **E**, NS1, NS2A (nucleotides 2293 to 4125)] and JEV30 [containing a SmaI site followed by JE cDNA encoding 30 aa **E**, NS1, NS2A, NS2B (nucleotides 2293 to 4512)].

DETD HeLa cell monolayers were prepared in 35 mm diameter dishes and infected with vaccinia viruses (**m**.o.i. of 2) or JEV (**m**.o.i. of 5) before radiolabeling. At 16 h post infection, cells were pulse labeled with medium containing ^{35}S-Met and chased. . .

DETD Recombinant vP825 encoded the capsid protein C, structural protein precursor **prM**, the structural glycoprotein **E**, the nonstructural glycoprotein NS1, and the nonstructural protein NS2A (McAda et al., 1987). Recombinant vP829 encoded the putative 15 aa **signal sequence** preceding the amino-terminus of **prM**, as well as **prM**, and **E** (McAda et al., 1987). Recombinant vP857 contained a cDNA encoding the 30 aa hydrophobic carboxy-terminus of **E**, followed by NS1 and NS2A. Recombinant vP864 contained a cDNA encoding the same proteins as vP857 with the addition of NS2B. In recombinants vP825 and vP829 a potential vaccinia virus early transcription termination signal in **E** (TTTTTGT; nucleotides 1399-1405) was modified to TCTTTGT without altering the aa sequence. This change was made in an attempt to increase the level of expression of **E** since this sequence has been shown to increase transcription termination in in vitro transcription assays (Yuen et al., 1987).

DETD **E** and **prM** Were Properly Processed When Expressed BY Recombinant Vaccinia Viruses

DETD Pulse-chase experiments demonstrate that proteins identical in size to **E** were synthesized in cells infected with all recombinant vaccinia viruses containing the **E** gene (Table 3). In the case of cells infected with JEV, vP555 and vP829, an **E** protein that migrated slower in SDS-PAGE was also detected in the culture fluid harvested from the infected cells (Table 3). This extracellular form of **E** produced by JEV- and vP555-infected cells contained mature N-linked glycans (Mason, 1989; Mason et al., 1991), as confirmed for the extracellular forms of **E** produced by vP829-infected cells. Interestingly, vP825, which contained the C coding region in addition to **prM** and **E** specified the synthesis of **E** in a form that is not released into the extracellular fluid (Table 3). Immunoprecipitations prepared from radiolabeled vaccinia-infected cells using a MAb specific for **M** (and **prM**) revealed that **prM** was synthesized in cells infected with vP555, vP825, and vP829, and **M** was detected in the culture fluid of cells infected with vP555 or vP829 (Table 3).

DETD . . . fluids (Table 3). This result indicated that vP829 infected cells produced extracellular particles similar to the empty viral envelopes containing **E** and **M** which are observed in the culture fluids harvested from vP555 infected cells (FIG. 9).

DETD Pre-challenge sera pooled from selected animals in each group were tested for their ability to immunoprecipitate radiolabeled **E** and NS1. The results of these studies (Table 3) demonstrated that: (1) the following order of immune response to **E** vP829>vP555>vP825, (2) all viruses encoding NS1 and NS2A induced antibodies to NS1, and (3) all immune responses were increased by. . . sera collected from these animals (Table 4) confirmed the results of the immunoprecipitation analyses, showing that the immune response to **E** as demonstrated by RIP correlated well with these other serological tests (Table 4).

DETD TABLE 3

Characterization of proteins expressed by vaccinia recombinants and their immune responses

	vP555	vP829	vP825	vP857	vP864
Proteins expressed					
Intracellular	**prM,E** NS1	**prM,E**	**prM,E** NS1	NS1	NS1
secreted	**M,E**,NS1	**M,E**	NS1	NS1	NS1

Particle formation	+	+	-	-	-
Immune response					
single	**E**	**E**	NS1	NS1	NS1
double	**E**,NS1	**E**	**E**,NS1	NS1	NS1

single = single inoculation with 10^7 pfu vaccinia recombinants (ip)
double = two inoculations with 10^7 pfu vaccinia. . .

DETD . . . isolated and ligated to a SacI (JEV nucleotide 2125) to EagI fragment of JEV25 (containing the remaining two thirds of **E**, translation stop and T5NT) generating JEV36. JEV36 was transfected into vP866 (NYVAC) infected cells to generate the vaccinia recombinant vP923. . .

DETD Plasmid YFO containing YF cDNA encoding the carboxy-terminal 80% **prM**, **E** and amino-terminal 80% NS1 (nucleotides 537-3266) was derived by cloning an AvaI to NsiI fragment of YF cDNA (nucleotides 537-1658). . . and KpnI digested IBI25 (International Biotechnologies, Inc., New Haven, Conn.). Plasmid YF1 containing YF cDNA encoding C and amino-terminal 20% **prM** (nucleotides 119-536) was derived by cloning a RsaI to AvaI fragment of YF cDNA (nucleotides 166-536) and annealed oligos SP46. . . and YF nucleotides 119-165) into AvaI and HindIII digested IBI25. Plasmid YF3 containing YF cDNA encoding the carboxy-terminal 60% of **E** and amino-terminal 25% of NS1 was generated by cloning an ApaI to BamHI fragment of YF cDNA (nucleotides 1604-2725) into. . . cDNA (nucleotides 4339-4940) into SacI and XbaI digested IBI25. Plasmid YF13 containing YF cDNA encoding the carboxy-terminal 25% of C, **prM** and amino-terminal 40% of **E** was derived by cloning a BalI to ApaI fragment of YF cDNA (nucleotides 384-1603) into ApaI and SmaI digested IBI25.

DETD . . . gene in YF1 (TTTTTCT nucleotides 263-269 and TTTTTGT nucleotides 269-275) to (SEQ ID NO:35) TTCTTCTTCTTGT creating plasmid YF1B, in the **E** gene in YF3 (nucleotides 1886-1893 TTTTTTGT to TTCTTTGT 189 aa from the carboxy-terminus and nucleotides 2429-2435 TTTTTGT to TTCTTGT 8. . . YF3C (nucleotides 1965-2725) was exchanged for the corresponding fragment of YF3B generating YF4 containing YF cDNA encoding the carboxy-terminal 60% **E** and amino-terminal 25% NS1 (nucleotides 1604-2725) with both mutagenized transcription termination signals. An ApaI to BamHI fragment from YF4 (nucleotides 1604-2725) was substituted for the equivalent region in YF0 creating plasmid YF6 containing YF cDNA encoding the carboxy-terminal 80% **prM**, **E** and amino-terminal 80% NS1 (nucleotides 537-3266) with both mutagenized transcription termination signals. Plasmid YF6 was digested with EcoRV within the. . .

DETD . . . mutagenesis described above was used to insert XhoI and ClaI sites preceding the ATG 17 aa from the carboxy-terminus of **E** (nucleotides 2402-2404) in plasmid YF3C creating YF5, to insert XhoI and ClaI sites preceding the ATG 19 aa from the carboxy-terminus of **prM** (nucleotides 917-919) in plasmid YF13 creating YF14, to insert an XhoI site preceding the ATG 23 aa from the carboxy-terminus of **E** (nucleotides 2384-2386) in plasmid YF3C creating plasmid YF25, and to insert an XhoI site and ATG (nucleotide 419) in plasmid. . .

DETD . . . YF5 (nucleotides 1604-2725) was exchanged for the corresponding region of YF0 creating YF7 containing YF cDNA encoding the carboxy-terminal 80% **prM**, **E** and amino-terminal 80% NS1 (nucleotides 537-3266) with XhoI and ClaI sites at 2402 (17 aa from the carboxy-terminus of **E**) and a mutagenized transcription termination signal at 2429-2435 (8 aa from the carboxy-terminus of **E**). The ApaI to BamHI fragment from YF25 (nucleotides 1604-2725) was exchanged for the corresponding region of YF0 generating YF26 containing YF cDNA encoding the carboxy-terminal 80% **prM**, **E** and amino-terminal 80% NS1 (nucleotides 537-3266) with an XhoI site at nucleotide 2384 (23 aa from the carboxy-terminus of **E**) and mutagenized transcription termination signal at 2428-2435 (8 aa from the carboxy-terminus of **E**).

DETD . . . YF14 (nucleotides 537-1603) was substituted for the corresponding region in YF6 generating YF15 containing YF cDNA encoding the carboxy-terminal 80% **prM**, **E** and amino-terminal 80% NS1

(nucleotides 537-3266) with XhoI and ClaI sites at nucleotide 917 (19 aa from the carboxy-terminus of **prM**) and two mutagenized transcription termination signals. YF6 was digested within IBI25 with EcoRV and within YF at nucleotide 537 with. . .

DETD . . . from YF12 encoding carboxy-terminal 20% NS1, NS2A and NS2B (nucleotides 3267-4569), XhoI to KpnI fragment from YF15 encoding 19 aa **prM, E** and amino-terminal 80% NS1 (nucleotides 917-3266) and XhoI-SmaI digested pHES4 were ligated generating YF23. An XhoI to BamHI fragment from YF26 encoding 23 aa **E**, amino-terminal 25% NS1 (nucleotides 2384-2725) was ligated to an XhoI to BamHI fragment from YF23 (containing the carboxy-terminal 75% NS1,. . .

DETD XhoI-SmaI digested pHES4 was ligated to a purified XhoI to KpnI fragment from YF7 encoding 17 aa **E** and amino-terminal 80% NS1 (nucleotides 2402-3266) plus a KpnI to SmaI fragment from YF10 encoding the carboxy-terminal 20% NS1 and NS2A (nucleotides 3267-4180) creating YF18. An XhoI to BamHI fragment from YF2 encoding C, **prM, E** and amino-terminal 25% NS1 (nucleotides 119-2725) was ligated to a XhoI to BamHI fragment of YF18 (containing the carboxy-terminal 75%. . . the origin of replication and vaccinia sequences) generating YF20. A XhoI to BamHI fragment from YF46 encoding 21 aa C, **prM, E** and amino-terminal 25% NS1 (nucleotides 419-2725) was ligated to the XhoI to BamHI fragment from YF18 generating YF47. Oligonucleotide SP46. . .

DETD Vero cell monolayers were infected with vaccinia virus for 1 hr (**m**.o.i.=10) before radiolabeling. After the absorption period the inoculum was removed and infected cells were overlaid with Met-free media (MEM) containing. . .

DETD HeLa cell monolayers were infected with vaccinia virus (**m**.o.i.=2) or YF17D (**m**.o.i.=4) before radiolabeling. At 38 hr post infection for YF17D or 16 hr post infection for vaccinia, cells were pulsed labeled. . .

DETD Radiolabeled cell lysates and culture fluids were harvested and the viral proteins were immunoprecipitated with monoclonal antibodies to YF **E** and NS1 and separated in SDS-containing polyacrylamide gels exactly as described by Mason (1989).

DETD Recombinant vP725 encoded the putative 17-aa **signal sequence** preceding the N terminus of the nonstructural protein NS1 and the nonstructural proteins NS1 and NS2A (Rice et al., 1985). Recombinant vP729 encoded the putative 19-aa **signal sequence** preceding the N terminus of **E, E**, NS1, NS2A and NS2B (Rice et al., 1985). Recombinant vP764 encoded C, **prM, E**, NS1, NS2A and NS2B (Rice et al., 1985). Recombinant vP766 encoded C, **prM, E**, NS1 and NS2A (Rice et al., 1985). Recombinant vP869 encoded the putative 21-aa **signal sequence** preceding the N terminus of the structural protein precursor **prM, prM E**, NS1 and NS2A (Rice et al., 1985).

DETD **E** Protein Expression By Recombinant Vaccinia Virus

DETD Pulse-chase experiments in HeLa cells demonstrated that a protein identical in size to YF17D **E** was synthesized in cells infected with vP869 and secreted into the culture fluid (Table 7). Under the same conditions of labeling, no intracellular or extracellular **E** was detected in cultures infected with vP766, vP729 or the control vaccinia virus vP457 (Table 7).

DETD Continuous label experiments in Vero cells demonstrated that a protein identical in size to the **E** protein expressed by vP869 was expressed in cultures infected with vP766 and vP729 (Table 7). These results suggest that the **E** protein produced by vP869 infected cells is present in a form in which it is more stable than the **E** protein expressed by vP766 or vP729. YF17D has previously been shown to produce a more labile **E** protein than other YF isolates (Cane et al. 1989).

DETD . . . immunization with YF17D. Pre-challenge sera pooled from selected animals in each group were tested for their ability to immunoprecipitate radiolabeled **E** and NS1 proteins and for the presence of Neut and HAI antibodies. As shown in Table 9 only vP869 and YF17D immunized mice responded to **E** protein, the response was increased by a second inoculation. Mice immunized twice with vP729, vP725 or vP766 produced antibody to. . .

DETD TABLE 7

Characterization of proteins expressed by vaccinia recombinants and YF17D

YF Proteins Expressed	17D	vP869	vP729	vP725	vP766	vP457
Intracellular	**E**,NS1	**E**	**E**,NS1	NS1	**E**,NS1	NONE
Secreted	**E**,NS1	**E**	NS1	NS1	NONE	NONE
Extracellular	YES	YES	No	NO	NO	NO
HA Activity						

DETD TABLE 9

Pre-challenge Radioimmunoprecipitation

	One Inoculation		Two Inoculations	
Immunizing Virus	Anti-**E**	Anti-NS1	Anti-**E**	Anti-NS1
vP457	-	-	-	-
vP725				+
vP729				+
vP766				+
vP869	+	-	++	-
17D	+	-	++	-

DETD A XhoI to SmaI fragment from YF47 (nucleotides 419-4180) containing YF CDNA encoding 21 amino acids C, **prM**, **E**, NS1, NS2A (with a base missing in NS1 nucleotide 2962) was ligated to XhoI-SmaI digested SPHA-H6 (HA region donor plasmid). . . (nucleotide 3262) and a 6700 bp fragment isolated (containing the plasmid origin of replication, vaccinia sequences, 21 amino acids C, **prM**, **E**, amino-terminal 3.5% NS1, carboxy-terminal 23% NS1, NS2A) and ligated to a SacI-Asp718 fragment from YF18 (containing the remainder of NS1. . . site in YF51 were removed using oligonucleotide-directed double-strand break mutagenesis (Mandecki, 1986) creating YF50 encoding YF 21 amino acids C, **prM**, **E**, NS1, NS2A in the HA locus donor plasmid. YF50 was transfected into vP866 (NYVAC) infected cells generating the recombinant vP984. . .

DETD . . . double-strand break mutagenesis creating YF49. Oligonucleotide-directed mutagenesis (Kunkel, 1985) was used to insert a SmaI site at the carboxy-terminus of **E** (nucleotide 2452) in YF4 creating YF16. An ApaI-SmaI fragment of YF49 (containing the plasmid origin of replication, vaccinia sequences and YF CDNA encoding 21 amino acids C, **prM**, and amino-terminal 43% **E**) was ligated to an ApaI-SmaI fragment from YF16 (nucleotides 1604-2452 containing the carboxy-terminal 57% **E**) generating YF53 containing 21 amino acids C, **prM**, **E** in the HA locus donor plasmid. YF53 was transfected into vP866 (NYVAC) infected cells generating the recombinant vP1003 (FIG. 19).. . .

DETD CLONING OF **DENGUE** TYPE 1 INTO A VACCINIA VIRUS DONOR PLASMID

DETD . . . digested pUC8. An EcoRI-HindIII fragment from DEN1 (nucleotides 2559-3745) and SacI-EcoRI fragment of DEN CDNA encoding the carboxy-terminal 36% of **E** and amino-terminal 16% NS1 (nucleotides 1447-2559, Mason et al., 1987B) were ligated to HindIII-SacI digested IBI24 (International Biotechnologies, Inc., New Haven, Conn.) generating DEN3 encoding the carboxy-terminal 64% **E** through amino-terminal 45% NS2A with a base missing in NS1 (nucleotide 2467).

DETD . . . an AvaI-SacI fragment of DEN CDNA (nucleotides 424-1447 Mason et al., 1987B) generating DEN4 encoding the carboxy-terminal 11 aa C, **prM** and amino-terminal 36% **E**.

DETD Plasmid DEN6 containing DEN cDNA encoding the carboxy-terminal 64% **E** and amino-terminal 18% NS1 (nucleotides 1447-2579 with nucleotide 2467

present Mason et al., 1987B) was derived by cloning a SacI XhoI. . . Biotechnologies, Inc., New Haven, Conn.). Plasmid DEN15 containing DEN cDNA encoding 51 bases of the DEN 5' untranslated region, C, **prM** and amino-terminal 36% **E** was derived by cloning a HindIII-SacI fragment of DEN cDNA (nucleotides 20-1447, Mason et al., 1987B) into HindIII-SacI digested IBI25.. . .

DETD . . . mutagenesis (Kunkel, 1985) was used to change potential vaccinia virus early transcription termination signals (Yuen et al., 1987) in the **prM** gene in DEN4 29 aa from the carboxy-terminus (nucleotides 822-828 TTTTTCT to TATTTCT) and 13 aa from the carboxy-terminus (nucleotides. . .

DETD . . . (nucleotide 4102) in plasmid DEN23 creating DEN24, to insert a SmaI site and ATG 15 aa from the carboxy-terminus of **E** in DEN7 (nucleotide 2348) creating DEN10, to insert an EagI and HindIII site at the carboxy-terminus of NS2B (nucleotide 4492). . .

DETD . . . DEN7 (nucleotides 1447-2579) was substituted for the corresponding region in DEN3 generating DEN19 containing DEN cDNA encoding the carboxy-terminal 64% **E** and amino-terminal 45% NS2A (nucleotides 1447-3745) with nucleotide 2467 present and the modified transcription termination signal (nucleotides 2448-2454). A XhoI-XbaI. . .

DETD . . . of the H6 promoter and DEN nucleotides 68-494) was ligated to a HindIII-PstI fragment from DEN47 (encoding the carboxy-terminal 83% **prM** and amino-terminal 36% of **E** nucleotides 494-1447 and plasmid origin of replication) generating DEN17 encoding C, **prM** and amino-terminal 36% **E** with part of the H6 promoter and EcoRV site preceding the amino-terminus of C. A HindIII-BglII fragment from DEN17 encoding the carboxy-terminal 13 aa C, **prM** and amino-terminal 36% **E** (nucleotides 370-1447) was ligated to annealed oligonucleotides SP111 and SP112 (containing a disabled HindIII sticky end, EcoRV site to -1. . . a BglII sticky end) creating DEN33 encoding the EcoRV site to -1 of the H6 promoter, carboxy-terminal 20 aa C, **prM** and amino-terminal 36% **E**.

DETD . . . digested pTP15 (Mason et al., 1991) was ligated to a SmaI-SacI fragment from DEN4 encoding the carboxy-terminal 11 aa C, **prM** and amino-terminal 36% **E** (nucleotides 377-1447) and SacI-EagI fragment from DEN3 encoding the carboxy-terminal 64% **E**, NS1 and amino-terminal 45% NS2A generating DENL. The SacI-XhoI fragment from DEN7 encoding the carboxy-terminal 64% **E** and amino-terminal 18% NS1 (nucleotides 1447-2579) was ligated to a BstEII-SacI fragment from DEN47 (encoding the carboxy-terminal 55% **prM** and amino-terminal 36% **E** (nucleotides 631-1447) and a BstEII-XhoI fragment from DENL (containing the carboxy-terminal 11 aa C, amino-terminal 45% **prM**, carboxy-terminal 82% NS1, carboxy-terminal 45% NS2A, origin of replication and vaccinia sequences) generating DEN8. A unique SmaI site (located between. . .

DETD An EcoRV-SacI fragment from DEN17 (positions -21 to -1 H6 promoter DEN nucleotides 68-1447) encoding C, **prM** and amino-terminal 36% **E**) was ligated to an EcoRV-SacI fragment of DEN8VC (containing vaccinia sequences, H6 promoter from -21 to -124, origin of replication and amino-terminal 64% **E**, NS1, amino-terminal 45% NS2A nucleotides 1447-3745) generating DEN18. A XhoI-EagI fragment from DEN25 encoding the carboxy-terminal 82% NS1 and NS2A. . . (nucleotides 2579-4102) was ligated to an XhoI-EagI fragment of DEN18 (containing the origin of replication, vaccinia sequences and DEN C **prM**, **E** and amino-terminal 18% NS1 nucleotides 68-2579) generating DEN26. An EcoRV-SacI fragment from DEN8VC (positions -21 to -1 H6 promoter DEN nucleotides 377-1447 encoding 11aaC, **prM** and amino-terminal 36% **E**) was ligated to an EcoRV-SacI fragment of DEN26 (containing the origin of replication, vaccinia sequences and DEN region encoding the carboxy-terminal 64% **E**, NS1 and NS2A with a base missing in NS1 at nucleotide 2894) generating DEN32. DEN32 was transfected into vP410 infected. . .

DETD . . . DEN10 (nucleotides 1447-2579) was substituted for the corresponding region in DEN3 generating DEN11 containing DEN cDNA encoding the carboxy-terminal 64% **E**, NS1 and amino-terminal 45% NS2A with a SmaI site and ATG 15 aa from the carboxy-terminus of **E**. A SmaI-EagI fragment from DEN11 (encoding the carboxy-terminal 15 aa **E**,

NS1 and amino terminal 49% NS2A nucleotides 2540-3745) was ligated to SmaI-EagI digested pTP15 generating DEN12.

DETD An EcoRV-XhoI fragment from DEN8VC (positions -21 to -1 H6 promoter DEN nucleotides 377-2579 encoding the carboxy-terminal 11 aa C, **prM E**, amino-terminal 18% NS1) was ligated to an EcoRV-XhoI fragment from DEN31 (containing the origin of replication, vaccinia sequences and DEN. . . An EcoRV-SacI fragment from DEN33 (positions -21 to -1 H6 promoter DEN nucleotides 350-1447 encoding the carboxy-terminal 20 aa C, **prM** and amino-terminal 36% **E**) and a SacI-XhoI fragment from DEN32 (encoding the carboxy-terminal 64% **E** and amino-terminal 18% NS1 nucleotides 1447-2579) were ligated to the EcoRV-SacI fragment from DEN31 described above generating DEN34. DEN34 was. . .

DETD . . . vaccinia recombinants vP962, vP955, vP867, vP452 (vaccinia control) or 100 µl of a 10% suspension of suckling mouse brain containing **dengue** type 1 Hawaii strain. Three weeks later sera were collected. One group of mice was re-inoculated and sera were collected. . .

DETD . . . twice with vP962 developed high levels of HAI antibodies, levels were equivalent to those obtained in animals immunized twice with **Dengue** type 1 Hawaii strain.

DETD Construction of Vaccinia Insertion Vector Containing DEN Type 1 20aaC, **prM, E**

DETD A 338 bp fragment encoding the carboxy-terminal 23% **E** (nucleotides 2055-2392, Mason et al., 1987b) TGA stop codon T5NT vaccinia early transcription termination signal (Yuen et al., 1987) and. . . and cloned into HindIII/BamHI digested IBI25 generating DEN36. DEN34 was digested with EcoRV (within the H6 promoter) and HindIII within **E** (DEN nucleotide 2061; Mason et al., 1987b) and a 1733 bp fragment (containing EcoRV to -1 H6 promoter, 20 aaC, **prM** and amino-terminal 77% **E**) was isolated. DEN36 was digested with HindIII and EclXI and a 331 bp fragment isolated (containing DEN nucleotides 2062-2392 TGA. . . generating plasmid DEN38. Plasmid DEN38 can be transfected into vaccinia infected cells to generate a recombinant encoding DEN 20 aaC, **prM** and **E**.

DETD This example describes the development of canarypox recombinant vCP107 encoding JEV 15aaC, **prM**, **E**, NS1, NS2A and a canarypox donor plasmid (JEVCPC5) encoding 15aaC, **prM**, **E**.

DETD Construction of Insertion Vector Containing JEV 15aaC, **prM**, **E**, NS1, NS2A

DETD Construction of pRW838 is illustrated below (FIG. 23). Oligonucleotides A through **E**, which overlap the translation initiation codon of the H6 promoter with the ATG of rabies G, were cloned into pUC9 as pRW737. Oligonucleotides A through **E** contain the H6 promoter, starting at NruI, through the HindIII site of rabies G followed by BglII. Sequences of oligonucleotides A through **E** are:

DETD . . . 62):

CTGAAATTATTTCATTATCGCGATATCCGTTAAGTTT
GTATCGTAATGGTTCCTCAGGCTCTCCTGTTTGT

B (SEQ ID NO: 63):

CATTACGATACAAACTTAACGGATATCGCGATAATGAAAT
AATTTCAG

C (SEQ ID NO: 64):

ACCCCTTCTGGTTTTTCCGTTGTGTTTTGGGAAATT
CCCTATTTACACGATCCCAGACAAGCTTAGATCTCAG

D (SEQ ID NO: 65):

CTGAGATCTAAGCTTGTCTGGGATCGTGTAAATAGGGAAT
TTCCCAAAACA

E (SEQ ID NO: 66):

CAACGGAAAAACCAGAAGGGGTACAAACAGGAGAGCCTGA
GGAAC

DETD The diagram of annealed oligonucleotides A through **E** is as follows:
##STR16##

DETD Oligonucleotides A through **E** were kinased, annealed (95° C. for 5 minutes, then cooled to room temperature), and inserted between the PvuII sites of. . .

DETD . . . promoter, plasmid origin of replication and C5 flanking arms isolated. Plasmid JEVL14VC containing JEV cDNA encoding 15 amino acids

C, prM, E, NS1, NS2A in a vaccinia virus donor plasmid (FIG. 1) (nucleotides 337-4125, FIGS. 17A and B) (SEQ ID NO:52) was. . . JEVCP1 was transfected into ALVAC infected primary CEF cells to generate the canarypox recombinant vCP107 encoding 15 amino acids C, **prM**, **E**, NS1, NS2A (FIG. 18).

DETD Construction of C5 Insertion Vector Containing JEV 15aac, **prM**, **E**

DETD . . . annealed oligonucleotides SP131 (SEQ ID NO:75) and SP132 (SEQ ID NO:76) (containing a SphI sticky end, T nucleotide completing the **E** coding region, translation stop, a vaccinia early transcription termination signal (AT5AT; Yuen and Moss, 1987), a second translation stop, and XbaI sticky end) generating plasmid JEVCP5 which encodes 15 amino acids C, **prM** and **E** under the control of the H6 promoter between C5 flanking arms. JEVCP5 can be transfected in ALVAC or ALVAC recombinant infected cells to generate a recombinant encoding JEV 15 aa C, **prM** and **E**. ##STR18##

DETD . . . and SmaI and ligated to a 3772 bp XhoI-SmaI fragment from YF51 (nucleotides 419-4180 encoding YF 21 amino acids C, **prM**, **E**, NS1, NS2A) generating YF52. The 6 bp corresponding to the unique XhoI site in UP52 were removed using oligonucleotide-directed double-strand. . . YFCP3. YFCP3 was transfected into ALVAC infected primary CEF cells to generate the canarypox recombinant vCP127 encoding 21 aa C, **prM**, **E**, NS1, NS2A (FIG. 19).

DETD Construction of C3 Insertion vector Containing YFV 21 aa C, **prM**, **E**

DETD . . . 8344 bp fragment isolated (containing the plasmid origin of replication, canarypox DNA and YF CDNA encoding 21 amino acids C, **prM**, and amino-terminal 57% **E**) and ligated to a ApaI to SmaI fragment from YF16 (nucleotides 1604-2452 containing the carboxy-terminal 43% **E**) generating YF54. The 6 bp corresponding to the unique XhoI site in YF54 were removed as described above creating YFCP4 containing YF cDNA encoding 21 amino acids C, **prM**, and **E**. YFCP4 can be transfected into ALVAC or ALVAC recombinant infected cells to generate a recombinant encoding YFV 21 aa C, **prM**, **E**.

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1. A recombinant avipox virus comprising DNA coding for Japanese **encephalitis virus** protein **M** or a precursor to protein **M**, and Japanese **encephalitis virus** protein **E**, NS1 and NS2A, in a nonessential region of the avipox genome.

4. A recombinant avipox virus comprising DNA from Japanese **encephalitis virus** (JEV) in a nonessential region of the avipox genome, wherein the DNA comprises the part of the JEV open reading frame extending from **prM** to NS2a.

. . . and an avipox virus according to claim 1, wherein the composition is effective to induce a protective response against Japanese **encephalitis virus** in a host animal.

. . . and an avipox virus according to claim 3, wherein the composition is effective to induce a protective response against Japanese **encephalitis virus** in a host animal.

. . . and an avipox virus according to claim 4, wherein the composition is effective to induce a protective response against Japanese **encephalitis virus** in a host animal.

. . . and an avipox virus according to claim 7, wherein the composition is effective to induce a protective response against Japanese **encephalitis virus** in a host animal.

L14 ANSWER 14 OF 15 USPATFULL on STN
96:38606 **Flavivirus** recombinant poxvirus vaccine.
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Virogenetics Corporation, Troy, NY, United States (U.S. corporation)
US 5514375 19960507
APPLICATION: US 1991-714687 19910613 (7) <--
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB What is described is a recombinant poxvirus, such as vaccinia virus, fowlpox virus and canarypox virus, containing foreign DNA from **flavivirus**, such as Japanese **encephalitis virus**, **yellow fever virus** and **Dengue** virus. In a preferred embodiment, the recombinant poxvirus generates an extracellular particle containing **flavivirus E** and **M** proteins capable of inducing neutralizing antibodies, hemagglutination-inhibiting antibodies and protective immunity against **flavivirus** infection. What is also described is a vaccine containing the recombinant poxvirus for inducing an immunological response in a host animal inoculated with the vaccine.
CLM What is claimed is:
1. A recombinant vaccinia virus comprising DNA coding for Japanese **encephalitis virus** protein **M** or a precursor to protein **M**, and Japanese **encephalitis virus** proteins **E**, NS1 and NS2A, in a nonessential region of the vaccinia genome.

2. The recombinant vaccinia virus of claim 1 wherein regions C7L-K1L, J2R, B13R+B14R, A26L, A56R, and I4L have been deleted therefrom.

3. The recombinant vaccinia virus of claim 1 wherein the open reading frames for the thymidine kinase gene, the hemorrhagic region, the A type

inclusion body region, the hemagglutinin gene, the host range gene region, and the large subunit, ribonucleotide reductase have been deleted therefrom.

4. An immunological composition which induces an immunological response in a host animal inoculated with said composition comprising a carrier and a recombinant vaccinia virus as claimed in claim 1.

5. An immunological composition which induces an immunological response in a host animal inoculated with said composition comprising a carrier and a recombinant vaccinia virus as claimed in claim 2.

6. A recombinant vaccinia virus as in claim 2 wherein the poxvirus is a NYVAC recombinant vaccinia virus.

7. An immunological composition which induces an immunological response in a host animal inoculated with said composition comprising a carrier and a recombinant vaccinia virus as claimed in claim 6.

8. An immunological composition which induces an immunological response in a host animal inoculated with said composition comprising a carrier and a recombinant vaccinia virus as claimed in claim 3.

9. A recombinant vaccinia virus as in claim 3 wherein the vaccinia virus is a NYVAC recombinant vaccinia virus.

10. A vaccine which induces an immunological response in a host animal inoculated with said vaccine, said vaccine comprising a carrier and a recombinant vaccinia virus as claimed in claim 7.

11. A recombinant vaccinia virus as in claim 1 which is selected from the group consisting of vP650, vP555, and vP908.

12. A vaccine which induces an immunological response in a host animal inoculated with said vaccine, said vaccine comprising a carrier and a recombinant vaccinia virus as claimed in claim 1.

13. A vaccine which induces an immunological response in a host animal inoculated with said vaccine, said vaccine comprising a carrier and a recombinant vaccinia virus as claimed in claim 2.

14. A vaccine for which induces an immunological response in a host animal inoculated with said vaccine, said vaccine comprising a carrier and a recombinant vaccinia virus as claimed in claim 3.

15. A recombinant vaccinia virus wherein regions C7L-K1L, J2R, B13R+B14R, A26L, A56R, and I4L have been deleted therefrom, and further comprising DNA from Japanese **encephalitis virus** in a non-essential region of the vaccinia genome.

16. A recombinant vaccinia virus as in claim 15 wherein the vaccinia virus is a NYVAC recombinant vaccinia virus.

17. A recombinant vaccinia virus wherein the open reading frames for the thymidine kinase gene, the hemorrhagic region, the A type inclusion body region, the hemagglutinin gene, the host range gene region, and the large subunit, ribonucleotide reductance have been deleted therefrom, and further comprising DNA from Japanese **encephalitis virus** in a non-essential region of the vaccinia genome.

18. A recombinant vaccinia virus as in claim 17 wherein the vaccinia virus is a NYVAC recombinant vaccinia virus.

19. A recombinant vaccinia virus as claimed in claim 17 which is: vP923.

20. A recombinant vaccinia virus comprising DNA from Japanese

encephalitis virus in a nonessential region of the vaccinia genome wherein the DNA codes for a precursor to Japanese **encephalitis virus** protein **M** and Japanese **encephalitis virus** proteins C, **E**, NS1 and NS2A; or, the DNA codes for Japanese **encephalitis virus** proteins NS1 and NS2A; or, the DNA codes for Japanese **encephalitis virus** proteins NS1, NS2A and NS2B.

21. A recombinant vaccinia virus as claimed in claim 20 which is: vP825, vP857 or vP864.

22. A recombinant vaccinia virus as in claim 6 which is vP908.

TI **Flavivirus** recombinant poxvirus vaccine

AI US 1991-714687 19910613 (7) <--

AB What is described is a recombinant poxvirus, such as vaccinia virus, fowlpox virus and canarypox virus, containing foreign DNA from **flavivirus**, such as Japanese **encephalitis virus**, **yellow fever virus** and **Dengue** virus. In a preferred embodiment, the recombinant poxvirus generates an extracellular particle containing **flavivirus E** and **M** proteins capable of inducing neutralizing antibodies, hemagglutination-inhibiting antibodies and protective immunity against **flavivirus** infection. What is also described is a vaccine containing the recombinant poxvirus for inducing an immunological response in a host. . .

SUMM . . . and using the same. More in particular, the invention relates to recombinant poxvirus, which virus expresses gene products of a **flavivirus** gene, and to vaccines which provide protective immunity against **flavivirus** infections.

SUMM . . . sequence to be inserted into the virus, particularly an open reading frame from a non-pox source, is placed into an **E**. coli plasmid construct into which DNA homologous to a section of DNA of the poxvirus has been inserted. Separately, the. . . flanking a region of pox DNA containing a nonessential locus. The resulting plasmid construct is then amplified by growth within **E**. coli bacteria (Clewell, 1972) and isolated (Clewell et al., 1969; Maniatis et al., 1986).

SUMM Second, the isolated plasmid containing the DNA gene sequence to be inserted is transfected into a cell culture, **e**.g. chick embryo fibroblasts, along with the poxvirus. Recombination between homologous pox DNA in the plasmid and the viral genome respectively. . .

SUMM The family **Flaviviridae** comprises approximately 60 arthropod-borne viruses that cause significant public health problems in both temperate and tropical regions of the world. . . developed against some of these agents, there has been a recent surge in the study of the molecular biology of **flaviviruses** in order to produce recombinant vaccines to the remaining viruses, most notably **dengue** (Brandt, 1988).

SUMM **Flavivirus** proteins are encoded by a single long translational open reading frame (ORF) present in the positive-strand genomic RNA. The genes. . . end of the genome followed by the nonstructural glycoprotein NS1 and the remaining nonstructural proteins (Rice et al., 1985). The **flavivirus** virion contains an **envelope** glycoprotein, **E**, a **membrane** protein, **M**, and a capsid protein, C. In the case of Japanese **encephalitis virus** (JEV), virion preparations usually contain a small amount of the glycoprotein precursor to the **membrane** protein, **prM** (Mason et al., 1987). Within JEV-infected cells, on the other hand, the **M** protein is present almost exclusively as the higher molecular weight **prM** protein (Mason et al., 1987A; Shapiro et al., 1972).

SUMM Studies that have examined the protective effect of passively administered monoclonal antibodies (MAbs) specific for each of the three **flavivirus** glycoproteins (**prM**, **E**, NS1) have demonstrated that immunity to each of these antigens results in partial or complete protection from lethal viral challenge. Monoclonal antibodies to **E** can provide protection from infection by Japanese **encephalitis virus** (JEV) (Kimura-Kuroda et al., 1988; Mason et al., 1989), **dengue** type 2 virus (Kaufman et al., 1987) and **yellow fever virus** (YF) (Gould et al., 1986). In most cases, passive protection has been correlated with

the ability of these E MAbs to neutralize the virus in vitro. Recently, Kaufman et al. (1989) have demonstrated that passive protection can also be. . . attenuate viral infection by blocking virus binding to target cells. Passive protection experiments using MAbs to the NS1 protein of **yellow fever virus** (Schlesinger et al., 1985; Gould et al., 1986) and **dengue** type 2 virus (Henchal et al., 1988) have demonstrated that antibodies to this nonstructural glycoprotein can protect animals from lethal. . .

SUMM . . . of NS1 immunity to protect the host from infection comes from direct immunization experiments in which NS1 purified from either **yellow fever virus**-infected cells (Schlesinger et al., 1985, 1986) or **dengue** type 2 virus-infected cells (Schlesinger et al., 1987) induced protective immunity from infection with the homologous virus.

SUMM Although significant progress has been made in deriving the primary structure of these three **flavivirus** glycoprotein antigens, less is known about their three-dimensional structure. The ability to produce properly folded, and possibly correctly assembled, forms. . . NS1-based vaccines, dimerization of NS1 (Winkler et al., 1988) may be required to elicit the maximum protective response. For the **E** protein, correct folding is probably required for eliciting a protective immune response since **E** protein antigens produced in **E**. coli (Mason et al., 1989) and the authentic **E** protein prepared under denaturing conditions (Wengler et al., 1989b) failed to induce neutralizing antibodies. Correct folding of the **E** protein may require the coordinated synthesis of the **prM** protein, since these proteins are found in heterodimers in the cell-associated forms of West Nile virus (Wengler et al., 1989a). The proper folding of **E** and the assembly of **E** and **prM** into viral particles may require the coordinated synthesis of the NS1 protein, which is coretained in an early compartment of the secretory apparatus along with immature forms of **E** in JEV-infected cells (Mason, 1989).

SUMM Attempts to produce recombinant **flavivirus** vaccines based on the **flavivirus** glycoproteins has met with some success, although protection in animal model systems has not always correlated with the predicted production. . .

SUMM . . . a vaccinia recombinant containing the region of JEV encoding 65 out of the 127 amino acids of C, all of **prM**, all of **E**, and 59 out of the 352 amino acids of NS1. Haishi et al. (1989) reported a vaccinia recombinant containing Japanese encephalitis sequences encoding 17 out of the 167 amino acids of **prM**, all of **E** and 57 out of the 352 amino acids of NS1.

SUMM Deubel et al. (1988) reported a vaccinia recombinant containing the **dengue**-2 coding sequences for all of C, all of **prM**, all of **E** and 16 out of the 352 amino acids of NS1.

SUMM Zhao et al. (1987) reported a vaccinia recombinant containing the **dengue**-4 coding sequences for all of C, all of **prM**, all of **E**, all of NS1, and all of NS2A. Bray et al. (1989) reported a series of vaccinia recombinants containing the **dengue**-4 coding sequences for (i) all of C, all of **prM** and 416 out of the 454 amino acids of **E**, (ii) 15 out of the 167 amino acids of **prM** and 416 out of the 454 amino acids of **E**, (iii) 18 amino acids of influenza A virus hemagglutinin and 416 out of the 454 amino acids of **E**, and (iv) 71 amino acids of respiratory syncytial virus G glycoprotein and 416 out of the 454 amino acids of **E**.

SUMM Despite these attempts to produce recombinant **flavivirus** vaccines, the proper expression of the JEV **E** protein by the vaccinia recombinants has not been satisfactorily obtained. Although Haishi et al. (1989) demonstrated cytoplasmic expression of JEV **E** protein by their vaccinia recombinant, the distribution was different from that observed in JEV infected cells. Yasuda et al. (1990) detected expression of JEV **E** protein by their vaccinia recombinant on the cell surface. Recombinant viruses that express the **prM** and **E** protein protected mice from approximately 10 LD_{50} of challenge virus. Yasuda et al. (1990) elicited anti-JEV immune responses as well as protection but reactivity to a panel of **E** specific monoclonal antibodies exhibited differences from the reactivity observed in JEV infected cells.

SUMM **Dengue** type 2 structural proteins have been expressed by recombinant

vaccinia viruses (Deubel et al., 1988). Although these viruses induced the synthesis of the structural glycoprotein within infected cells, they neither elicited detectable anti-**dengue** immune responses nor protected monkeys from **dengue** infection. Several studies also have been completed on the expression of portions of the **dengue** type 4 structural and nonstructural proteins in vaccinia virus (Bray et al., 1989; Falgout et al., 1989; Zhao et al.,. . . the viral ORF extending from C to NS2A under the control of the P7.5 early-late promoter produced intracellular forms of **prM**, **E**, and NS1 but failed to induce the synthesis of extracellular forms of any of the structural proteins, even though a. . . this recombinant virus (Bray et al., 1989; Zhao et al., 1987). Additional recombinant viruses that contained several forms of the **dengue** type 4 **E** gene with or without other structural protein genes have also been examined (Bray et al., 1989). Although several of these recombinant viruses were able to induce protection, they neither produced extracellular forms of **E** nor induced neutralizing antibodies.

SUMM It can thus be appreciated that provision of a **flavivirus** recombinant poxvirus which produces properly processed forms of **flavivirus** proteins, and of vaccines which provide protective immunity against **flavivirus** infections, would be a highly desirable advance over the current state of technology.

SUMM It is therefore an object of this invention to provide recombinant poxviruses, which viruses express properly processed gene products of **flavivirus**, and to provide a method of making such recombinant poxviruses.

SUMM It is an additional object of this invention to provide for the cloning and expression of **flavivirus** coding sequences in a poxvirus vector.

SUMM It is another object of this invention to provide a vaccine which is capable of eliciting **flavivirus** neutralizing antibodies, hemagglutination-inhibiting antibodies and protective immunity against **flavivirus** infection and a lethal **flavivirus** challenge.

SUMM In one aspect, the present invention relates to a recombinant poxvirus generating an extracellular **flavivirus** structural protein capable of inducing protective immunity against **flavivirus** infection. In particular, the recombinant poxvirus generates an extracellular particle containing **flavivirus** **E** and **M** proteins capable of eliciting neutralizing antibodies and hemagglutination-inhibiting antibodies. The poxvirus is advantageously a vaccinia virus or an avipox virus, such as fowlpox virus or canarypox virus. The **flavivirus** is advantageously Japanese **encephalitis virus**, **yellow fever virus** and **Dengue** virus.

SUMM According to the present invention, the recombinant poxvirus contains therein DNA from **flavivirus** in a nonessential region of the poxvirus genome for expressing in a host **flavivirus** structural protein capable of release to an extracellular medium. In particular, the DNA contains Japanese **encephalitis virus** coding sequences that encode a precursor to structural protein **M**, structural protein **E**, and nonstructural proteins NS1 and NS2A. More in particular, the recombinant poxvirus contains therein DNA from **flavivirus** in a nonessential region of the poxvirus genome for expressing a particle containing **flavivirus** structural protein **E** and structural protein **M**.

SUMM . . . with the vaccine, said vaccine including a carrier and a recombinant poxvirus containing, in a nonessential region thereof, DNA from **flavivirus**.

SUMM More in particular, the recombinant viruses express portions of the **flavivirus** ORF extending from **prM** to NS2B. Biochemical analysis of cells infected with the recombinant viruses showed that the recombinant viruses specify the production of properly processed forms of all three **flavivirus** glycoproteins--**prM**, **E**, and NS1. The recombinant viruses induced synthesis of extracellular particles that contained fully processed forms of the **M** and **E** proteins. Furthermore, the results of mouse immunization studies demonstrated that the induction of neutralizing antibodies and high levels of protection were associated with the ability of the immunizing recombinant viruses to produce extracellular particles containing the two structural **membrane** proteins.

DRWD FIG. 7 shows a comparison by SDS-PAGE analysis of the cell lysate **E** proteins produced by JEV infection and infection with the recombinant

vaccinia viruses vP650, vP555, vP658 and vP583;

DRWD FIG. 8 shows a comparison by SDS-PAGE analysis of the culture fluid **E** proteins produced by JEV infection and infection with the recombinant vaccinia viruses vP650, vP555, vP658 and vP583;

DRWD FIG. 9 shows a comparison by sucrose gradient analysis of the forms of the **E** protein found in the culture fluid harvested from JEV infected cells and cells infected with vaccinia recombinants vP555 and vP650;

DETD . . . The resulting plasmid, pJEV1, contained the viral ORF extending from the SacI site (nucleotide 1904) in the last third of **E** through the BalI site (nucleotide 3909) two amino acid residues (aa) into the predicted N terminus of NS2B (FIG. 1).

DETD . . . containing a XhoI sticky end, a SmaI site, the last 15 aa of C, and first 9 aa of JEV **prM** with a sticky HindIII end) were ligated to a HindIII-SacI fragment of JEV cDNA (nucleotides 184-1904), and XhoI-SacI digested vector. . . the viral ORF extending between the methionine (Met) codon (nucleotides 115-117) occurring 15 aa preceding the predicted N terminus of **prM** and the SacI site (nucleotide 1904) found in the last third of **E** (FIG. 1).

DETD . . . pJEV5, contained the viral ORF extending between the Met codon (nucleotides 592-594) occurring 25 aa preceding the N terminus of **E** and the SacI site (nucleotide 1904) found in the last third of **E** (FIG. 1).

DETD . . . (Kunkel, 1985) was used to change a potential vaccinia virus early transcription termination signal (Yuen et al., 1987) in the **E** gene of pJEV2 (TTTTTGT; nucleotides 1088-1094) to TCTTTGT, creating plasmid pJEV22 (FIG. 2). The same change was performed on pJEV5. . .

DETD . . . resulting plasmid, pJEV7, contained the viral ORF extending between the SacI site (nucleotide 1904) found in the last third of **E** and the last codon of NS2B (nucleotide 4296) (FIG. 2). SmaI-EagI digested pTP15 was purified and ligated to the purified. . .

DETD BHK or VERO cell monolayers were prepared in 35 mm diameter dishes and infected with vaccinia viruses (**m**.o.i. of 2) or JEV (**m**.o.i. of 5) and incubated for 11 hr (vaccinia) or 16 hr (JEV) before radiolabeling. At 11 hr or 16 hr. . .

DETD Four different vaccinia virus recombinants were constructed that expressed portions of the JEV coding region extending from **prM** through NS2B. The JEV cDNA sequences contained in these recombinant viruses are shown in FIG. 4. In all four recombinant. . .

DETD Recombinant vP555 encodes the putative 15 aa **signal sequence** preceding the N terminus of the structural protein precursor **prM**, the structural glycoprotein **E**, the nonstructural glycoprotein NS1, and the nonstructural protein NS2A (McAda et al., 1987). Recombinant vP583 encodes the putative **signal sequence** preceding the N terminus of **E**, **E**, NS1, and NS2A (McAda et al., 1987). Recombinant vP650 contains a cDNA encoding the same proteins as vP555 with the. . . vP583 with the addition of NS2B. In recombinants vP650 and vP658, a potential vaccinia virus early transcription termination signal in **E** (TTTTTGT; nucleotides 1087-1094) was modified to TCTTTGT without altering the aa sequence. This change was made in an attempt to increase the level of expression of **E** and NS1, since this sequence has been shown to increase transcription termination in in vitro transcription assays (Yuen et al.,. . .

DETD . . . noted that recombinants vP555, vP583, and vP650 had a deletion from within the HindIII C fragment through HindIII N and **M** and into HindIII K. This same deletion was observed in the vP425 parental virus. Interestingly, these viruses were less cytopathic. . .

DETD . . . lysate (FIG. 5) or culture fluid (FIG. 6) prepared from each cell layer were immunoprecipitated, and then either mock digested (**M**), digested with endo H (H), or digested with PNGase F (F), prior to SDS-PAGE analysis.

DETD . . . production by all four recombinants, suggesting that the potential vaccinia early transcriptional termination signal present near the end of the **E** coding region in vP555 and vP583 did not significantly reduce the amount of NS1 produced relative to vP650 or vP658. . .

DETD **E** and **prM** were Properly Processed when Expressed by Recombinant

vaccinia viruses

DETD FIGS. 7 and 8 show a comparison of the **E** protein produced by JEV infection or infection with the recombinant vaccinia viruses. BHK cells were infected with JEV or recombinant. . . lysate (FIG. 7) or culture fluid (FIG. 8) prepared from each cell layer were immunoprecipitated, and then either mock digested (**M**), digested with endo H (H), or digested with PNGase F (F), prior to SDS-PAGE analysis.

DETD The data from the pulse-chase experiments depicted in FIGS. 7 and 8 demonstrate that proteins identical in size to **E** were synthesized in cells infected with all recombinant vaccinia viruses containing the **E** gene. However, the **E** protein was only released from cells infected with vaccinia viruses that contained the region of the viral ORF encoding **prM**, **E**, NS1, and NS2A (vP555 and vP650; see FIGS. 4, 7 and 8). Endoglycosidase sensitivity (FIGS. 7 and 8) revealed that both the intracellular and extracellular forms of the **E** protein synthesized by cells infected with the vaccinia recombinants were glycosylated; the cell-associated forms of **E** were endo H sensitive, whereas the extracellular forms were resistant to endo H digestion.

DETD Immunoprecipitates prepared from radiolabeled vaccinia-infected cells using a MAb specific for **M** (and **prM**) revealed that **prM** was synthesized in cells infected with vP555 and vP650. Cells infected with either of these recombinant vaccinia viruses produced cellular forms of **prM** that were identical in size to the **prM** protein produced by JEV-infected cells, and a **M** protein of the correct size was detected in the culture fluid of cells infected with these two viruses.

DETD The extracellular fluid harvested from cells infected with vP555 and vP650 contained forms of **E** that migrated with a peak of hemagglutinating activity in sucrose density gradients. Interestingly, this hemagglutinin migrated similarly to the slowly. . . found in the culture fluid of JEV-infected cells (FIG. 9). Furthermore, these same fractions contained the fully processed form of **M**, demonstrating that vP555- and vP650-infected cells produced a particle that contained both of the structural **membrane** proteins of JEV. These particles probably represent empty JEV envelopes, analogous to the 22 nm hepatitis B virus particles found. . .

DETD Recombinant vaccinia virus vP555 produced **E**- and **M**-containing extracellular particles that behaved like empty viral envelopes. The ability of this recombinant virus to induce the synthesis of extracellular. . .

DETD The recombinant viruses described herein contain portions of the JEV ORF that encode the precursor to the structural protein **M**, the structural protein **E**, and nonstructural proteins NS1, NS2A, and NS2B. The **E** and NS1 proteins produced by cells infected with these recombinant viruses underwent proteolytic cleavage and N-linked carbohydrate addition in a. . . proteins produced by cells infected with JEV. These data further demonstrate that the proteolytic cleavage and N-linked carbohydrate addition to **E** and NS1 do not require **flavivirus** nonstructural proteins located 3' to NS2A in the viral genome (Bray et al., 1989; Deubel et al., 1988; Falgout et. . .

DETD . . . the portion of the ORF inserted in the recombinant vaccinia viruses had a significant effect on the late-stage processing of **prM** and **E**, but not on the fate of NS1. All recombinant viruses that encoded NS1 produced mature extracellular forms of this protein,. . . from transfected cells (Fan et al., 1990). On the other hand, only two of the four recombinants that contained the **E** protein coding region produced extracellular forms of **E**. These two recombinants, vP555 and vP650, differed from the remaining recombinants in that they contained the **prM** coding region in addition to **E**, NS1, and NS2A. The findings that extracellular forms of **E** were produced only by viruses containing the coding regions for both **E** and **prM** and that the extracellular forms of **E** were associated with **M** suggest that the simultaneous synthesis of **prM** and **E** is a requirement for the formation of particles that are targeted for the extracellular fluid.

DETD . . . the co-migration of the rabbit immunoglobulin heavy chain with the radiolabeled viral antigens, and to permit clear separation of the **E** and the NS1' proteins. Neutralization tests were performed on

heat inactivated sera (20 min. at 56° C.) as described (Fesh et. . .

DETD . . . virus, two viruses (vP555 and vP658) were selected for in-depth challenge studies. vP555 induced the synthesis of extracellular forms of **E**, whereas vP658 did not produce any extracellular forms of **E**, but contained additional cDNA sequences encoding the NS2B protein. In the challenge experiments several dilutions of challenge virus were tested,. . . dose of JEV. The analysis demonstrated that: (1) only those animals immunized with vP555 showed a strong immune response to **E**, and (2) a second inoculation resulted in a significant increase in reactivity to the **E** protein (FIG. 10).

DETD . . . induce neutralizing antibodies may be related to the fact that vP555 produces an extracellular particulate form of the structural proteins **E** and **M**. This SHA-like particle probably represents an empty JEV **envelope** that contains **E** and **M** folded and assembled into a configuration very similar to that found in the infectious JEV particle. Recombinants vP555 and vP650. . . assembly of viral envelopes. Other investigators (see above) have not been able to detect the production of extracellular forms of **E** by cells expressing all three structural proteins (C, **prM**, and **E**) in the presence or absence of NS1 and NS2A. The inability of their recombinant viruses to produce particles similar to. . . that the C protein produced in the absence of a genomic RNA interferes with the proper assembly of the viral **membrane** proteins. Alternatively, an incompletely processed form of C similar to that detected by Nowak et al. (1989) in in vitro translation experiments, could prevent release of the structural **membrane** proteins from the cells expressing the C gene.

DETD . . . from Bethesda Research Laboratories, Gaithersburg, Md., New England Biolabs, Beverly, Mass.; and Boehringer Mannheim Biochemicals, Indianapolis, Ind. Klenow fragment of **E**. coli polymerase was obtained from Boehringer Mannheim Biochemicals. BAL-31 exonuclease and phage T4 DNA ligase were obtained from New England. . .

DETD . . . NcoI site (pos. 172,253) were removed by digestion of pSD419 with NcoI/SmaI followed by blunt ending with Klenow fragment of **E**. coli polymerase and ligation generating plasmid pSD476. A vaccinia right flanking arm was obtained by digestion of pSD422 with HpaI. . . ID NO:6/SEQ ID NO:7) ##STR3## generating pSD479. pSD479 contains an initiation codon (underlined) followed by a BamHI site. To place **E**. coli Beta-galactosidase in the B13-B14 (u) deletion locus under the control of the u promoter, a 3.2 kb BamHI fragment. . .

DETD . . . at the pUC/vaccinia junction was destroyed by digestion of pSD478 with EcoRI followed by blunt ending with Klenow fragment of **E**. coli polymerase and ligation, generating plasmid pSD478E$^-$. pSD478E$^-$ was digested with BamHI and HpaI and ligated with annealed synthetic oligonucleotides. . .

DETD . . . xbaI within vaccinia sequences (pos. 137,079) and with HindIII at the pUC/vaccinia junction, then blunt ended with Klenow fragment of **E**. coli polymerase and ligated, resulting in plasmid pSD483. To remove unwanted vaccinia DNA sequences to the right of the A26L. . . digestion with BglII (pos. 140,136) and with EcoRI at the pUC/vaccinia junction, followed by blunt ending with Klenow fragment of **E**. coli polymerase and ligation. The resulting plasmid was designated pSD489. The 1.8 kb ClaI (pos. 137,198)/EcoRV (pos. 139,048) fragment from. . .

DETD A 3.3 kb BglII cassette containing the **E**. coli Beta-galactosidase gene (Shapira et al., 1983) under the control of the vaccinia 11 kDa promoter (Bertholet et al., 1985;. . .

DETD A 3.2 kb BglII/BamHI (partial) cassette containing the **E**. coli Beta-galactosidase gene (Shapira et al., 1983) under the control of the vaccinia 11 kDa promoter (Bertholet et al., 1985;. . .

DETD . . . were removed from the pUC/vaccinia junction by digestion of pSD466 with EcoRI/BamHI followed by blunt ending with Klenow fragment of **E**. coli polymerase and ligation. Recombination between vP708 and pSD467 resulted in recombinant vaccinia deletion mutant, vP723, which was isolated as. . .

DETD . . . SphI and religated, forming pSD451. In pSD451, DNA sequences to the left of the SphI site (pos. 27,416) in HindIII **M** are removed

(Perkus et al., 1990). pSD409 is HindIII **M** cloned into pUC8.

DETD To provide a substrate for the deletion of the [C7L-K1L] gene cluster from vaccinia, **E**. coli Beta-galactosidase was first inserted into the vaccinia M2L deletion locus (Guo et al., 1990) as follows. To eliminate the. . . unique BglII site inserted into the M2L deletion locus as indicated above. A 3.2 kb BamHI (partial)/BglII cassette containing the **E**. coli Beta-galactosidase gene (Shapira et al., 1983) under the control of the 11 kDa promoter (Bertholet et al., 1985) was. . .

DETD . . . deleted for vaccinia genes [C7L-K1L] was assembled in pUC8 cut with SmaI, HindIII and blunt ended with Klenow fragment of **E**. coli polymerase. The left flanking arm consisting of vaccinia HindIII C sequences was obtained by digestion of pSD420 with XbaI (pos. 18,628) followed by blunt ending with Klenow fragment of **E**. coli polymerase and digestion with BglII (pos. 19,706). The right flanking arm consisting of vaccinia HindIII K sequences was obtained. . .

DETD . . . coding sequences, pSD518 was digested with BamHI (pos. 65,381) and HpaI (pos. 67,001) and blunt ended using Klenow fragment of **E**. coli polymerase. This 4.8 kb vector fragment was ligated with a 3.2 kb SmaI cassette containing the **E**. coli Beta-galactosidase gene (Shapira et al., 1983) under the control of the vaccinia 11 kDa promoter (Berthoist et al., 1985;. . .

DETD . . . mutagenized expression cassette contained within pRW837 was derived by digestion with HindIII and EcoRI, blunt-ended using the Klenow fragment of **E**. coli DNA polymerase in the presence of 2mM dNTPs, and inserted into the SmaI site of pSD 513 to yield. . .

DETD . . . into pRW843 (containing the measles HA gene). Plasmid pRW843 was first digested with NotI and blunt-ended with Klenow fragment of **E**. coli DNA polymerase in the presence of 2 mM dNTPs. The resulting plasmid, pRW857, therefore contains the measles virus F. . .

DETD . . . and AccI fragment of JEV2 (Mason et al., 1991) containing the plasmid origin and JEV cDNA encoding the carboxy-terminal 40% **prM** and amino-terminal two thirds of **E** (nucleotides 696 to 2215), generating plasmid JEV20 containing JE sequences from the ATG of C through the SacI site (nucleotide 2215) found in the last third of **E**.

DETD . . . 1) in which TTTTTGT nucleotides 1399 to 1405 were changed to TCTTTGT), containing JE sequences from the last third of **E** through the first two amino acids of NS2B (nucleotides 2215 to 4220), the plasmid origin and vaccinia sequences, was ligated. . .

DETD . . . end] generated plasmid JEV25 which contains JE cDNA extending from the SacI site (nucleotide 2215) in the last third of **E** through the carboxy-terminus of **E**. The SacI-EagI fragment from JEV25 was ligated to the SacI-EagI fragment of JEV8 (containing JE cDNA encoding 15 aa C, **prM** and amino-terminal two thirds of **E** nucleotides 432 to 2215, the plasmid origin and vaccinia sequences) yielding plasmid JEV26. A unique SmaI site preceding the ATG. . .

DETD . . . or HpaI-HindIII fragment from JEV7 (FIG. 2) yielded JEV29 [containing a SmaI site followed by JE cDNA encoding 30 aa **E**, NS1, NS2A (nucleotides 2388 to 4220)] and JEV30 [containing a SmaI site followed by JE cDNA encoding 30 aa **E**, NS1, NS2A, NS2B (nucleotides 2388 to 4607)].

DETD HeLa cell monolayers were prepared in 35 mm diameter dishes and infected with vaccinia viruses (**m**.o.i. of 2) or JEV (**m**.o.i. of 5) before radiolabeling. At 16 h post infection, cells were pulse labeled with medium containing ^{35} S-Met and chased. . .

DETD Recombinant vP825 encoded the capsid protein C, structural protein precursor **prM**, the structural glycoprotein **E**, the nonstructural glycoprotein NS1, and the nonstructural protein NS2A (McAda et al., 1987). Recombinant vP829 encoded the putative 15 aa **signal sequence** preceding the amino-terminus of **prM**, as well as **prM**, and **E** (McAda et al., 1987). Recombinant vP857 contained a cDNA encoding the 30 aa hydrophobic carboxy-terminus of **E**, followed by NS1 and NS2A. Recombinant vP864 contained a cDNA encoding the same proteins as vP857 with the addition of NS2B. In recombinants vP825 and vP829 a potential vaccinia virus early transcription termination signal in **E** (TTTTTGT; nucleotides 1399-1405) was modified to TCTTTGT without altering the aa sequence. This change was made in an attempt to increase the level of

expression of **E** since this sequence has been shown to increase transcription termination in in vitro transcription assays (Yuen et al., 1987).

DETD **E** and **prM** Were Properly Processed When Expressed By Recombinant Vaccinia Viruses

DETD Pulse-chase experiments demonstrate that proteins identical in size to **E** were synthesized in cells infected with all recombinant vaccinia viruses containing the **E** gene (Table 3). In the case of cells infected with JEV, vP555 and vP829, an **E** protein that migrated slower in SDS-PAGE was also detected in the culture fluid harvested from the infected cells (Table 3). This extracellular form of **E** produced by JEV- and vP555-infected cells contained mature N-linked glycans (Mason, 1989; Mason et al., 1991), as confirmed for the extracellular forms of **E** produced by vP829-infected cells. Interestingly, vP825, which contained the C coding region in addition to **prM** and **E** specified the synthesis of **E** in a form that is not released into the extracellular fluid (Table 3). Immunoprecipitations prepared from radiolabeled vaccinia-infected cells using a MAb specific for **M** (and **prM**) revealed that **prM** was synthesized in cells infected with vP555, vP825, and vP829, and **M** was detected in the culture fluid of cells infected with vP555 or vP829 (Table 3).

DETD . . . fluids (Table 3). This result indicated that vP829 infected cells produced extracellular particles similar to the empty viral envelopes containing **E** and **M** which are observed in the culture fluids harvested from vP555 infected cells (FIG. 9).

DETD Pre-challenge sera pooled from selected animals in each group were tested for their ability to immunoprecipitate radiolabeled **E** and NS1. The results of these studies (Table 3) demonstrated that: (1) the following order of immune response to **E** vP829>vP555>vP825, (2) all viruses encoding NS1 and NS2A induced antibodies to NS1, and (3) all immune responses were increased by. . . sera collected from these animals (Table 4) confirmed the results of the immunoprecipitation analyses, showing that the immune response to **E** as demonstrated by RIP correlated well with these other serological tests (Table 4).

DETD TABLE 3

Characterization of proteins expressed by vaccinia recombinants and their immune responses

	vP555	vP829	vP825	vP857	vP864
Proteins expressed					
Intracellular	**prM, E** NS1	**prM, E** NS1	**prM, E** NS1	NS1	NS1
secreted	**M, E,** NS1	**M, E**	NS1	NS1	NS1
Particle formation	+	+	-	-	-
Immune response					
single	**E**	**E**	NS1	NS1	NS1
double	**E,** NS1	**E**	**E,** NS1 NS1		NS1

single = single inoculation with 10^7 pfu vaccinia recombinants (ip)
double = two inoculations with 10^7 pfu. . .

DETD . . . isolated and ligated to a SacI (JEV nucleotide 2215) to EagI fragment of JEV25 (containing the remaining two thirds of **E**, translation stop and T5NT) generating JEV36. JEV36 was transfected into vP866 (NYVAC) infected cells to generate the vaccinia recombinant vP923. . .

DETD Plasmid YF0 containing YF cDNA encoding the carboxy-terminal 80% **prM**, **E** and amino-terminal 80% NS1 (nucleotides 537-3266) was derived by cloning an AvaI to NsiI fragment of YF cDNA (nucleotides 537-1658). . . and KpnI digested IBI25 (International Biotechnologies, Inc., New Haven, Conn.). Plasmid YF1 containing YF cDNA encoding C and amino-terminal 20% **prM** (nucleotides 119-536) was derived by cloning a

KsaI to AvaI fragment of YF cDNA (nucleotides 160-330) and annealed oligos SP46. . . and YF nucleotides 119-165) into AvaI and HindIII digested IBI25. Plasmid YF3 containing YF cDNA encoding the carboxy-terminal 60% of **E** and amino-terminal 25% of NS1 was generated by cloning an ApaI to BamHI fragment of YF cDNA (nucleotides 1604-2725) into. . . cDNA (nucleotides 4339-4940) into SacI and xbaI digested IBI25. Plasmid YF13 containing YF cDNA encoding the carboxy-terminal 25% of C, **prM** and amino-terminal 40% of **E** was derived by cloning a BalI to ApaI fragment of YF cDNA (nucleotides 384-1603) into ApaI and SmaI digested IBI25.

DETD . . . gene in YF1 (TTTTTCT nucleotides 263-269 and TTTTTGT nucleotides 269-275) to TTCTTCTTCTTGT (SEQ ID NO:35) creating plasmid YF1B, in the **E** gene in YF3 (nucleotides 1886-1893 TTTTTTGT to TTCTTTGT 189 aa from the carboxy-terminus and nucleotides 2429-2435 TTTTTGT to TTCTTGT 8. . . YF3C (nucleotides 1965-2725) was exchanged for the corresponding fragment of YF3B generating YF4 containing YF cDNA encoding the carboxy-terminal 60% **E** and amino-terminal 25% NS1 (nucleotides 1604-2725) with both mutagenized transcription termination signals. An ApaI to BamHI fragment from YF4 (nucleotides 1604-2725) was substituted for the equivalent region in YF0 creating plasmid YF6 containing YF cDNA encoding the carboxy-terminal 80% **prM**, **E** and amino-terminal 80% NS1 (nucleotides 537-3266) with both mutagenized transcription termination signals. Plasmid YF 6 was digested with EcoRV within. . .

DETD . . . mutagenesis described above was used to insert XhoI and ClaI sites preceding the ATG 17 aa from the carboxy-terminus of **E** (nucleotides 2402-2404) in plasmid YF3C creating YF5, to insert XhoI and ClaI sites preceding the ATG 19 aa from the carboxy-terminus of **prM** (nucleotides 917-919) in plasmid YF13 creating YF14, to insert an XhoI site preceding the ATG 23 aa from the carboxy-terminus of **E** (nucleotides 2384-2386) in plasmid YF3C creating plasmid YF25, and to insert an XhoI site and ATG (nucleotide 419) in plasmid. . .

DETD . . . YF5 (nucleotides 1604-2725) was exchanged for the corresponding region of YF0 creating YF7 containing YF cDNA encoding the carboxy-terminal 80% **prM**, **E** and amino-terminal 80% NS1 (nucleotides 537-3266) with XhoI and ClaI sites at 2402 (17 aa from the carboxy-terminus of **E**) and a mutagenized transcription termination signal at 2429-2435 (8 aa from the carboxy-terminus of **E**). The ApaI to BamHI fragment from YF25 (nucleotides 1604-2725) was exchanged for the corresponding region of YF0 generating YF26 containing YF cDNA encoding the carboxy-terminal 80% **prM**, **E** and amino-terminal 80% NS1 (nucleotides 537-3266) with an XhoI site at nucleotide 2384 (23 aa from the carboxy-terminus of **E**) and mutagenized transcription termination signal at 2428-2435 (8 aa from the carboxy-terminus of **E**).

DETD . . . YF14 (nucleotides 537-1603) was substituted for the corresponding region in YF6 generating YF15 containing YF cDNA encoding the carboxy-terminal 80% **prM**, **E** and amino-terminal 80% NS1 (nucleotides 537-3266) with XhoI and ClaI sites at nucleotide 917 (19 aa from the carboxy-terminus of **prM**) and two mutagenized transcription termination signals. YF6 was digested within IBI25 with EcoRV and within YF at nucleotide 537 with. . .

DETD . . . from YF12 encoding carboxy-terminal 20% NS1, NS2A and NS2B (nucleotides 3267-4569), XhoI to KpnI fragment from YF15 encoding 19 aa **prM**, **E** and amino-terminal 80% NS1 (nucleotides 917-3266) and XhoI-SmaI digested pHES4 were ligated generating YF23. An XhoI to BamHI fragment from YF26 encoding 23 aa **E**, amino-terminal 25% NS1 (nucleotides 2384-2725) was ligated to an XhoI to BamHI fragment from YF23 (containing the carboxy-terminal 75% NS1,. . .

DETD XhoI-SmaI digested pHES4 was ligated to a purified XhoI to KpnI fragment from YF7 encoding 17 aa **E** and amino-terminal 80% NS1 (nucleotides 2402-3266) plus a KpnI to SmaI fragment from YF10 encoding the carboxy-terminal 20% NS1 and NS2A (nucleotides 3267-4180) creating YF18. An XhoI to BamHI fragment from YF2 encoding C, **prM**, **E** and amino-terminal 25% NS1 (nucleotides 119-2725) was ligated to a XhoI to BamHI fragment of YF18 (containing the carboxy-terminal 75%. . . the origin of replication and vaccinia sequences) generating YF20. A XhoI to

BamHI fragment from YF40 encoding 21 aa C, **prM**, **E** and amino terminal 25% NS1 (nucleotides 419-2725) was ligated to the XhoI to BamHI fragment from YF18 generating YF47. Oligonucleotide SP46. . .

DETD Recombinant vP725 encoded the putative 17-aa **signal sequence** preceding the N terminus of the nonstructural protein NS1 and the nonstructural proteins NS1 and NS2A (Rice et al., 1985). Recombinant vP729 encoded the putative 19-aa **signal sequence** preceding the N terminus of **E**, **E**, NS1, NS2A and NS2B (Rice et al., 1985). Recombinant vP764 encoded C, **prM**, **E**, NS1, NS2A and NS2B (Rice et al., 1985). Recombinant vP766 encoded C, **prM**, **E**, NS1 and NS2A (Rice et al., 1985). Recombinant vP869 encoded the putative 21-aa **signal sequence** preceding the N terminus of the structural protein precursor **prM**, **prM** **E**, NS1 and NS2A (Rice et al., 1985).

DETD A XhoI to SmaI fragment from YF47 (nucleotides 419-4180) containing YF cDNA encoding 21 amino acids C, **prM**, **E**, NS1, NS2A (with a base missing in NS1 nucleotide 2962) was ligated to XhoI-SmaI digested SPHA-H6 (HA region donor plasmid). . . (nucleotide 3262) and a 6700 bp fragment isolated (containing the plasmid origin of replication, vaccinia sequences, 21 amino acids C, **prM**, **E**, amino-terminal 3.5% NS1, carboxy-terminal 23% NS1, NS2A) and ligated to a SacI-Asp718 fragment from YF18 (containing the remainder of NS1. . . site in YF51 were removed using oligonucleotide-directed double-strand break mutagenesis (Mandecki, 1986) creating YF50 encoding YF 21 amino acids C, **prM**, **E**, NS1, NS2A in the HA locus donor plasmid. YF50 was transfected into vP866 (NYVAC) infected cells generating the recombinant vP984. . .

DETD . . . double-strand break mutagenesis creating YF49. Oligonucleotide-directed mutagenesis (Kunkel, 1985) was used to insert a SmaI site at the carboxy-terminus of **E** (nucleotide 2452) in YF4 creating YF16. An ApaI-SmaI fragment of YF49 (containing the plasmid origin of replication, vaccinia sequences and YF cDNA encoding 21 amino acids C, **prM**, and amino-terminal 43% **E**) was ligated to an ApaI-SmaI fragment from YF16 (nucleotides 1604-2452 containing the carboxy-terminal 57% **E**) generating YF53 containing 21 amino acids C, **prM**, **E** in the HA locus donor plasmid. YF53 was transfected into vP866 (NYVAC) infected cells generating the recombinant vP1003 (FIG. 18).. . .

DETD CLONING OF **DENGUE** TYPE 1 INTO A VACCINIA VIRUS DONOR PLASMID

DETD . . . digested pUC8. An EcoRI-HindIII fragment from DEN1 (nucleotides 2559-3745) and SacI-EcoRI fragment of DEN cDNA encoding the carboxy-terminal 36% of **E** and amino-terminal 16% NS1 (nucleotides 1447-2559, Mason et al., 1987B) were ligated to HindIII-SacI digested IBI24 (International Biotechnologies, Inc., New Haven, Conn.) generating DEN3 encoding the carboxy-terminal 64% **E** through amino-terminal 45% NS2A with a base missing in NS1 (nucleotide 2467).

DETD . . . an AvaI-SacI fragment of DEN cDNA (nucleotides 424-1447 Mason et al., 1987B) generating DEN4 encoding the carboxy-terminal 11 aa C, **prM** and amino-terminal 36% **E**.

DETD Plasmid DEN6 containing DEN cDNA encoding the carboxy-terminal 64% **E** and amino-terminal 18% NS1 (nucleotides 1447-2579 with nucleotide 2467 present Mason et al., 1987B) was derived by cloning a SacI-XhoI. . . Biotechnologies, Inc., New Haven, Conn.). Plasmid DEN15 containing DEN cDNA encoding 51 bases of the DEN 5' untranslated region, C, **prM** and amino-terminal 36% **E** was derived by cloning a HindIII-SacI fragment of DEN cDNA (nucleotides 20-1447, Mason et al., 1987B) into HindIII-SacI digested IBI25.. . .

DETD . . . mutagenesis (Kunkel, 1985) was used to change potential vaccinia virus early transcription termination signals (Yuen et al., 1987) in the **prM** gene in DEN4 29 aa from the carbox-f-terminus (nucleotides 822-828 TTTTTCT to TATTTCT) and 13 aa from the carboxy-terminus (nucleotides. . .

DETD . . . (nucleotide 4102) in plasmid DEN23 creating DEN24, to insert a SmaI site and ATG 15 aa from the carboxy-terminus of **E** in DEN7 (nucleotide 2348) creating DEN10, to insert an EagI and HindIII site at the carboxy-terminus of NS2B (nucleotide 4492). . .

DETD . . . DEN7 (nucleotides 1447-2579) was substituted for the

corresponding region in DEN3 generating DEN11 containing DEN cDNA encoding the carboxy-terminal 64% **E** and amino-terminal 45% NS2A (nucleotides 1447-3745) with nucleotide 2467 present and the modified transcription termination signal (nucleotides 2448-2454). A XhoI-XbaI. . .

DETD . . . of the H6 promoter and DEN nucleotides 68-494) was ligated to a HindIII-PstI fragment from DEN47 (encoding the carboxy-terminal 83% **prM** and amino-terminal 36% of **E** nucleotides 494-1447 and plasmid origin of replication) generating DEN17 encoding C, **prM** and amino-terminal 36% **E** with part of the H6 promoter and EcoRV site preceding the amino-terminus of C. A HindIII-BglII fragment from DEN17 encoding the carboxy-terminal 13 aa C, **prM** and amino-terminal 36% **E** (nucleotides 370-1447) was ligated to annealed oligonucleotides SPl11 and SPl12 (containing a disabled HindIII sticky end, ECoRV site to -1. . . . a BglII sticky end) creating DEN33 encoding the EcoRV site to -1 of the H6 promoter, carboxy-terminal 20 aa C, **prM** and amino-terminal 36% **E**.

DETD . . . digested pTP15 (Mason et al., 1991) was ligated to a SmaI-SacI fragment from DEN4 encoding the carboxy-terminal 11 aa C, **prM** and amino-terminal 36% **E** (nucleotides 377-1447) and SacI-EagI fragment from DEN3 encoding the carboxy-terminal 64% **E**, NS1 and amino-terminal 45% NS2A generating DENL. The SacI-XhoI fragment from DEN7 encoding the carboxy-terminal 64% **E** and amino-terminal 18% NS1 (nucleotides 1447-2579) was ligated to a BstEII-SacI fragment from DEN47 (encoding the carboxy-terminal 55% **prM** and amino-terminal 36% **E** (nucleotides 631-1447) and a BstEII-XhoI fragment from DENL (containing the carboxy-terminal 11 aa C, amino-terminal 45% **prM**, carboxy-terminal 82% NS1, carboxy-terminal 45% NS2A, origin of replication and vaccinia sequences) generating DENS. A unique SmaI site (located between. . .

DETD An EcoRV-SacI fragment from DEN17 (positions -21 to -1 H6 promoter DEN nucleotides 68-1447) encoding C, **prM** and amino-terminal 36% **E**) was ligated to an EcoRV-SacI fragment of DEN8VC (containing vaccinia sequences, H6 promoter from -21 to -124, origin of replication and amino-terminal 64% **E**, NS1, amino-terminal 45% NS2A nucleotides 1447-3745) generating DEN18. A XhoI-EagI fragment from DEN25 encoding the carboxy-terminal 82% NS1 and NS2A. . . (nucleotides 2579-4102) was ligated to an XhoI-EagI fragment of DEN18 (containing the origin of replication, vaccinia sequences and DEN C **prM**, **E** and amino-terminal 18% NS1 nucleotides 68-2579) generating DEN26. An EcoRV-SacI fragment from DEN8VC (positions -21 to -1 H6 promoter DEN nucleotides 377-1447 encoding 11aaC, **prM** and amino-terminal 36% **E**) was ligated to an EcoRV-SacI fragment of DEN26 (containing the origin of replication, vaccinia sequences and DEN region encoding the carboxy-terminal 64% **E**, NS1 and NS2A with a base missing in NS1 at nucleotide 2894) generating DEN32. DEN 32 was transfected into vP410. . .

DETD . . . DEN10 (nucleotides 1447-2579) was substituted for the corresponding region in DEN3 generating DEN11 containing DEN cDNA encoding the carboxy-terminal 64% **E**, NS1 and amino-terminal 45% NS2A with a SmaI site and ATG 15 aa from the carboxy-terminus of **E**. A SmaI-EagI fragment from DEN11 (encoding the carboxy-terminal 15 aa **E**, NS1 and amino-terminal 45% NS2A nucleotides 2348-3745) was ligated to SmaI-EagI digested pTP15 generating DEN12.

DETD An EcoRV-XhoI fragment from DEN8VC (positions -21 to -1 H6 promoter DEN nucleotides 377-2579 encoding the carboxy-terminal 11 aa C, **prM E**, amino-terminal 18% NS1) was ligated to an EcoRV-XhoI fragment from DEN31 (containing the origin of replication, vaccinia sequences and DEN. . . An EcoRV-SacI fragment from DEN33 (positions -21 to -1 H6 promoter DEN nucleotides 350-1447 encoding the carboxy-terminal 20 aa C, **prM** and amino-terminal 36% **E**) and a SacI-XhoI fragment from DEN32 (encoding the carboxy-terminal 64% **E** and amino-terminal 18% NS1 nucleotides 1447-2579) were ligated to the EcoRV-SacI fragment from DEN31 described above generating DEN34. DEN34 was. . .

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1. A recombinant vaccinia virus comprising DNA coding for Japanese **encephalitis virus** protein **M** or a precursor to protein **M**, and Japanese **encephalitis virus** proteins **E**, NS1 and NS2A, in a nonessential region of the vaccinia genome.

. . . virus wherein regions C7L-K1L, J2R, B13R+B14R, A26L, A56R, and I4L have been deleted therefrom, and further comprising DNA from Japanese **encephalitis virus** in a non-essential region of the vaccinia genome.

. . . host range gene region, and the large subunit, ribonucleotide reductance have been deleted therefrom, and further comprising DNA from Japanese **encephalitis virus** in a non-essential region of the vaccinia genome.

20. A recombinant vaccinia virus comprising DNA from Japanese **encephalitis virus** in a nonessential region of the vaccinia genome wherein the DNA codes for a precursor to Japanese **encephalitis virus** protein **M** and Japanese **encephalitis virus** proteins C, **E**, NS1 and NS2A; or, the DNA codes for Japanese **encephalitis virus** proteins NS1 and NS2A; or, the DNA codes for Japanese **encephalitis virus** proteins NS1, NS2A and NS2B.

L14 ANSWER 15 OF 15 USPATFULL on STN
96:16887 NYVAC vaccinia virus recombinants comprising heterologous inserts.
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APPLICATION: US 1993-105483 19930812 (8) <--
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB What is described is a modified vector, such as a recombinant poxvirus, particularly recombinant vaccinia virus, having enhanced safety. The modified recombinant virus has nonessential virus-encoded genetic functions inactivated therein so that virus has attenuated virulence. In one embodiment, the genetic functions are inactivated by deleting an open reading frame encoding a virulence factor. In another embodiment, the genetic functions are inactivated by insertional inactivation of an open reading frame encoding a virulence factor. What is also described is a vaccine containing the modified recombinant virus having nonessential virus-encoded genetic functions inactivated therein so that the vaccine has an increased level of safety compared to known recombinant virus vaccines.

CLM What is claimed is:

1. A recombinant vaccinia virus wherein regions C7L.-K1L, J2R, B13R+B14R, A26L, A56R and I4L have been deleted therefrom, and further comprising exogenous coding DNA from a non-vaccinia source in a nonessential region of the vaccinia genome.

2. A recombinant vaccinia virus wherein the open reading frames for the thymidine kinase gene, the hemorrhagic region, the A type inclusion body region, the hemagglutinin gene, the host range gene region, and, the large subunit, ribonucleotide reductase have been deleted therefrom, and further comprising exogenous coding DNA from a non-vaccinia source in a nonessential region of the vaccinia genome.

3. A recombinant vaccinia virus as claimed in claim 2 wherein the non-vaccinia source is selected from the group consisting of rabies virus, Hepatitis B virus, **yellow fever virus**, **Dengue** virus, pseudorabies virus, Epstein-Barr virus, herpes simplex virus, simian immunodeficiency virus, equine herpes virus, bovine herpes virus, bovine viral diarrhea virus, human cytomegalovirus, canine parvovirus, equine influenza virus, feline leukemia virus, feline herpes virus, Hantaan virus, C. tetani, avian influenza virus, mumps virus and Newcastle Disease virus.

4. A recombinant vaccinia virus as claimed in claim 3, wherein the non-vaccinia source is rabies virus and the recombinant vaccinia virus is vP879 or vP999.

5. A recombinant vaccinia virus as claimed in claim 3, wherein the non-vaccinia source is Hepatitis B virus and the recombinant vaccinia virus is vP856, vP896, vP897, vP858, vP891, vP932, vP975, vP930, vP919, vP941 or vP944.

6. A recombinant vaccinia virus as claimed in claim 3, wherein the non-vaccinia source is **yellow fever virus** and the recombinant vaccinia virus is vP766, vP764, vP869, vP729, vP725, vP997, or vP984.

7. A recombinant vaccinia virus as claimed in claim 3, wherein the non-vaccinia source is **Dengue** virus and the recombinant vaccinia virus is vP867, vP962 or vP955.

8. A recombinant vaccinia virus as claimed in claim 3, wherein the non-vaccinia source is pseudorabies virus and the recombinant vaccinia virus is vP881, vP883, vP900, vP912, vP925, vP915 or vP916.

9. A recombinant vaccinia virus as claimed in claim 3, wherein the non-vaccinia source is Epstein-Barr virus and the recombinant vaccinia virus is vP941 or vP944.

10. A recombinant vaccinia virus as claimed in claim 3, wherein the non-vaccinia source is herpes simplex virus and the recombinant vaccinia virus is vP914.

11. A recombinant vaccinia virus as claimed in claim 3, wherein the non-vaccinia source is simian immunodeficiency virus and the recombinant vaccinia virus is vP873, vP948, vP943, vP942, vP952, vP948, vP1042, vP1071, vP943, vP942, vP952 or vP1050.

12. A recombinant vaccinia virus as claimed in claim 3 wherein the non-vaccinia source is equine herpes virus and the recombinant vaccinia virus is vP1043, vP1025 or vP956.

13. A recombinant vaccinia virus as claimed in claim 3 wherein the non-vaccinia source is bovine herpes virus and the recombinant vaccinia virus is vP1051, vP1074, vP1073, vP1083, vP1087 or vP1079.

14. A recombinant vaccinia virus as claimed in claim 3 wherein the non-vaccinia source is bovine viral diarrhea virus and the recombinant vaccinia virus is vP972, vP1017 or vP1097.

15. A recombinant vaccinia virus as claimed in claim 3 wherein the non-vaccinia source is human cytomegalovirus and the recombinant vaccinia virus is vP1001.

16. A recombinant vaccinia virus as claimed in claim 3 wherein the non-vaccinia source is canine parvovirus and the recombinant vaccinia virus is vP998 or vP999.

17. A recombinant vaccinia virus as claimed in claim 3 wherein the non-vaccinia source is equine influenza virus and the recombinant vaccinia virus is vP961 or vP1063.

18. A recombinant vaccinia virus as claimed in claim 3 wherein the non-vaccinia source is feline leukemia virus and the recombinant vaccinia virus is vP1011.

19. A recombinant vaccinia virus as claimed in claim 3 wherein the non-vaccinia source is Hantaan virus and the recombinant vaccinia virus is vp882, vP950 or vP951.

20. A recombinant vaccinia virus as claimed in claim 3 wherein the non-vaccinia source is C. tetani and the recombinant vaccinia virus is vp1075.

21. An immunological composition for inducing an immunological response in a host inoculated with the composition, said composition comprising a carrier and a recombinant virus as claimed in any one of claims 2, 33, 44, 5, 6, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20.

22. A method for expressing a gene product in a cell cultured in vitro, which method comprises introducing into the cell a modified recombinant virus as claimed in claim 2.

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SUMM . . . sequence to be inserted into the virus, particularly an open reading frame from a non-pox source, is placed into an **E**. coli plasmid construct into which DNA homologous to a section of DNA of the poxvirus has been inserted. Separately, the. . . flanking a region of pox DNA containing a nonessential locus. The resulting plasmid construct is then amplified by growth within **E**. coli bacteria (Clewell, 1972) and isolated (Clewell et al., 1969; Maniatis et al., 1982).

SUMM Second, the isolated plasmid containing the DNA gene sequence to be inserted is transfected into a cell culture, **e**.g. chick embryo fibroblasts, along with the poxvirus. Recombination between homologous

pox DNA in the plasmid and the viral genome respectively. . .

SUMM Cowpox virus (Brighton red strain) produces red (hemorrhagic) pocks on the chorioallantoic **membrane** of chicken eggs. Spontaneous deletions within the cowpox genome generate mutants which produce white pocks (Pickup et al., 1984). The. . .

DRWD FIG. 26 shows the nucleotide sequence of FeLV-B **Envelope** Gene (SEQ ID NO:310);

DETD . . . from Bethesda Research Laboratories, Gaithersburg, Md., New England Biolabs, Beverly, Mass.; and Boehringer Mannheim Biochemicals, Indianapolis, Ind. Klenow fragment of **E**. coli polymerase was obtained from Boehringer Mannheim Biochemicals. BAL-31 exonuclease and phage T4 DNA ligase were obtained from New England. . .

DETD . . . NcoI site (pos. 172,253) were removed by digestion of pSD419 with NcoI/SmaI followed by blunt ending with Klenow fragment of **E**. coli polymerase and ligation generating plasmid pSD476. A vaccinia right flanking arm was obtained by digestion of pSD422 with HpaI. . . ID NO:6/SEQ ID NO:7) ##STR3## generating pSD479. pSD479 contains an initiation codon (underlined) followed by a BamHI site. To place **E**. coli Beta-galactosidase in the B13-B14 (u) deletion locus under the control of the u promoter, a 3.2 kb BamHI fragment. . .

DETD . . . at the pUC/vaccinia junction was destroyed by digestion of pSD478 with EcoRI followed by blunt ending with Klenow fragment of **E**. coli polymerase and ligation, generating plasmid pSD478E$^-$. pSD478E$^-$ was digested with BamHI and HpaI and ligated with annealed synthetic oligonucleotides. . .

DETD . . . XbaI within vaccinia sequences (pos. 137,079) and with HindIII at the pUC/vaccinia junction, then blunt ended with Klenow fragment of **E**. coli polymerase and ligated, resulting in plasmid pSD483. To remove unwanted vaccinia DNA sequences to the right of the A26L. . . digestion with BglII (pos. 140,136) and with EcoRI at the pUC/vaccinia junction, followed by blunt ending with Klenow fragment of **E**. coli polymerase and ligation. The resulting plasmid was designated pSD489. The 1.8 kb ClaI (pos. 137,198)/EcoRV (pos. 139,048) fragment from. . .

DETD A 3.3 kb BglII cassette containing the **E**. coli Beta-galactosidase gene (Shapira et al., 1983) under the control of the vaccinia 11 kDa promoter (Bertholet et al., 1985;. . .

DETD A 3.2 kb BglII/BamHI (partial) cassette containing the **E**. coli Beta-galactosidase gene (Shapira et al., 1983) under the control of the vaccinia 11 kDa promoter (Bertholet et al., 1985;. . .

DETD . . . were removed from the pUC/vaccinia junction by digestion of pSD466 with EcoRI/BamHI followed by blunt ending with Klenow fragment of **E**. coli polymerase and ligation. Recombination between vP708 and pSD467 resulted in recombinant vaccinia deletion mutant, vP723, which was isolated as. . .

DETD . . . SphI and religated, forming pSD451. In pSD451, DNA sequences to the left of the SphI site (pos. 27,416) in HindIII **M** are removed (Perkus et al., 1990). pSD409 is HindIII **M** cloned into pUC8.

DETD To provide a substrate for the deletion of the [C7L-K1L] gene cluster from vaccinia, **E**. coli Beta-galactosidase was first inserted into the vaccinia M2L deletion locus (Guo et al., 1990) as follows. To eliminate the. . .

DETD . . . unique BglII site inserted into the M2L deletion locus as indicated above. A 3.2 kb BamHI (partial)/BglII cassette containing the **E**. coli Beta-galactosidase gene (Shapira et al., 1983) under the control of the 11 kDa promoter (Bertholet et al., 1985) was. . .

DETD . . . deleted for vaccinia genes [C7L-K1L] was assembled in pUC8 cut with SmaI, HindIII and blunt ended with Klenow fragment of **E**. coli polymerase. The left flanking arm consisting of vaccinia HindIII C sequences was obtained by digestion of pSD420 with XbaI (pos. 18,628) followed by blunt ending with Klenow fragment of **E**. coli polymerase and digestion with BglII (pos. 19,706). The right flanking arm consisting of vaccinia HindIII K sequences was obtained. . .

DETD . . . coding sequences, pSD518 was digested with BamHI (pos. 65,381) and HpaI (pos. 67,001) and blunt ended using Klenow fragment of **E**. coli polymerase. This 4.8 kb vector fragment was ligated with a 3.2 kb SmaI cassette containing the **E**. coli Beta-galactosidase gene (Shapira

et al., 1989) under the control of the vaccinia 11 kDa promoter (Bertholet et al., 1985;. . .

DETD . . . al., 1989) was inserted into pCE13 by digesting pCE13 with SalI, filling in the sticky ends with Klenow fragment of **E**. coli DNA polymerase and digesting with HindIII. A HindIII-EcoRV fragment containing the H6 promoter sequence was then inserted into pCE13. . .

DETD . . . H6 promoted NDV-F cassette by cloning a HindIII fragment from pCE59 that had been filled in with Klenow fragment of **E**. coli DNA polymerase into the HpaI site of pCE71 to form pCE80. Plasmid pCE80 was completely digested with NdeI and. . .

DETD In NDV-infected cells, the F glycoprotein is anchored in the **membrane** via a hydrophobic transmembrane region near the carboxyl terminus and requires post-translational cleavage of a precursor, F_0, into two disulfide. . .

DETD . . . that immunoreactive proteins were presented on the infected cell surface. To determine that both proteins were presented on the plasma **membrane**, mono-specific rabbit sera were produced against vaccinia recombinants expressing either the F or HN glycoproteins. Indirect immunofluorescence using these sera. . .

DETD . . . mutagenized expression cassette contained within pRW837 was derived by digestion with HindIII and EcoRI, blunt-ended using the Klenow fragment of **E**. coli DNA polymerase in the presence of 2 mM dNTPs, and inserted into the SmaI site of pSD513 to yield. . .

DETD . . . into pRW843 (containing the measles HA gene). Plasmid pRW843 was first digested with NotI and blunt-ended witch Klenow fragment of **E**. coli DNA polymerase in the presence of 2 mM dNTPs. The resulting plasmid, pRW857, therefore contains the measles virus F. . .

DETD Immunoprecipitation. Immunoprecipitation reactions were performed as previously described (Taylor et al., 1990) using a guinea-pig anti measles serum (Whittaker **M**. A. Bioproducts, Walkersville, Md.).

DETD . . . The site-directed mutagenesis was done using MRSYN5 (SEQ ID NO:52) (5'-GCGAGCGAGGCCATGCATCGTGCGAATGGCCCC-3') and MRSYN6 (SEQ ID NO:53) (5'-GGGGGGACGCGCGGGTCTAGAAGGCCCCGCCTGGCGG-3') and selection on **E**. coli dut$^-$ ung$^-$ strain. CJ236 (International Biotechnologies, Inc., New Haven, Conn.). Mutagenesis was performed according to the protocols of Kunkel. . .

DETD . . . A 1.4 kb fragment containing the I3L promoter/PRV gp50 gene was isolated and blunt-ended using the Klenow fragment of the **E**. coli DNA polymerase in the presence of 2 mM dNTPs.

DETD Immunoprecipitation from NYVAC/PRV Recombinant Infected Cells. Vero cells were infected at an **m**.o.i. of 10 pfu per cell with the individual recombinant viruses, with the NYVAC parent virus, or were mock infected. After. . . were dissociated with RIPA buffer (1% NP-40, 1% Na-deoxycholate, 0.1% SDS, 0.01M methionine, 5 mM EDTA, 5 mM 2-mercapto-ethanol, 1 **m**/ml BSA, and 100 u/ml aprotinin). Samples analyzed with sheep anti-gpIII and a monoclonal specific for gp50 were lysed in 1×. . .

DETD Extraneous 3'-noncoding sequence was then eliminated from pGC10. This was accomplished by recircularizing the **E**. coli DNA polymerase I (Klenow fragment) filled-in 4,900 bp SalI-SmaI (partial) fragment of pGC10. The plasmid generated by this manipulation. . .

DETD Extraneous DNA was then eliminated. This was accomplished by cloning the **E**. coli DNA polymerase I (Klenow fragment) filled-in 6,000 bp HindIII-BamHI (partial) fragment of pGBCD1, containing the H6-promoted gB, gC and. . .

DETD Immunoprecipitation. Vero cells were infected at an **m**.o.i. of 10 pfu per cell with recombinant vaccinia virus, with the NYVAC parent virus (vP866) or were mock infected. After. . .

DETD . . . inserted individually into three different sites of the virus. The three HBV genes encode the following protein products: (1) HBV **M** protein, (referred to here as small pre S antigen, or spsAg), (2) HBV L protein (referred to here as large. . .

DETD The synthetic S1+S2 region was assembled in five double stranded sections A through **E** as indicated above using synthetic oligonucleotides, MPSYN290 through MPSYN308 (SEQ ID NO:90)-(SEQ ID NO:99), as set out below. Oligonucleotides ranged. . . within a

section were kinased before annealing of the section. Sequence of synthetic oligonucleotides used to construct sections A through **E** are given below. Only the coding strand is shown. Relevant restriction sites are noted. Initiation codons for S1 (section A), S2 (section C) and core (section **E**) are underlined. ##STR19##

DETD Construction of Insertion Vector Containing the Rabies G Gene. Construction of pRW838 is illustrated below. Oligonucleotides A through **E**, which overlap the translation initiation codon of the H6 promoter with the ATG of rabies G, were cloned into pUC9 as pRW737. Oligonucleotides A through **E** contain the H6 promoter, starting at NruI, through the HindIII site of rabies G followed by BglII. Sequences of oligonucleotides A through **E** (SEQ ID NO:109)-(SEQ ID NO. 113) are: ##STR22##

DETD The diagram of annealed oligonucleotides A through **E** is as follows: ##STR23##

DETD Oligonucleotides A through **E** were kinased, annealed (95° C. for 5 minutes, then cooled to room temperature), and inserted between the PvuII sites of. . .

DETD . . . stages of assembly of mature rabies virus particles, the glycoprotein component is transported from the golgi apparatus to the plasma **membrane** where it accumulates with the carboxy terminus extending into the cytoplasm and the bulk of the protein on the external surface of the cell **membrane**. In order to confirm that the rabies glycoprotein expressed in ALVAC-RG was correctly presented, immunofluorescence was performed on primary CEF. . .

DETD . . . MRC-5 cells--a diploid cell line derived from human fetal lung tissue (ATCC #CCL171). The initial inoculation was performed at an **m**.o.i. of 0.1 pfu per cell using three 60 mm dishes of each cell line containing 2×10^6 cells per dish. One. . .

DETD . . . parental canarypox virus, (b) ALVAC-RG, the recombinant expressing the rabies G glycoprotein or (c) vCP37, a canarypox recombinant expressing the **envelope** glycoprotein of feline leukemia virus. Inoculations were performed under ketamine anaesthesia. Each animal received at the same time: (1) 20. . .

DETD (**e**) Primary CEF cells.

DETD . . . electrophoresis the viral DNA band was visualized by staining with ethidium bromide. The DNA was then transferred to a nitrocellulose **membrane** and probed with a radiolabelled probe prepared from purified ALVAC genomic DNA.

DETD . . . seed 23 3.34

Vaccine Batch H	23	4.52
Vaccine Batch I	23	3.33
Vaccine Batch K	15	3.64
Vaccine Batch L	15	4.03
Vaccine Batch **M**	15	3.32
Vaccine Batch N	15	3.39
Vaccine Batch J	23	3.42

---

[a] Expressed as mouse LD_{50}

[b] Expressed as $\log_{10}$ $TCID_{50}$

DETD . . . 2.2

2.2

2.2

39 vCP37[d]

NT <1.2

<1.2

1.7

2.1

2.2

N.T.[g]

55 vCP37[d]

NT <1.2

<1.2

1.7

2.2

2.4
N.T.

37 ALVAC-RG[e]
2.2 <1.2
<1.2
3.2
3.5
3.5
3.2

53 ALVAC-RG[e]
2.2 <1.2
<1.2
3.6
3.6
3.6
3.4

38 ALVAC-RG[f]
2.7 <1.7
<1.7
3.2
3.8
3.6
N.T.

54 ALVAC-RG[f]
3.2 <1.7
<1.5
3.6

. . . 28 after primary vaccination

[c] Animals received 5.0 $\log_{10}$ $TCID_{50}$ of ALVAC

[d] Animals received 5.0 $\log_{10}$ $TCID_{50}$ of vCP37

[e] Animals received 5.0 $\log_{10}$ $TCID_{50}$ of ALVACRG

[f] Animals received 7.0 $\log_{10}$ $TCID_{50}$ of ALVACRG

[g] Not tested.

DETD TABLE 15

Inoculation of chimpanzees with ALVAC-RG

Weeks post-Inoculation	Animal 431 I.M.	Animal 457 S.C.
0	<8[a]	<8
1	<8	<8
2	8	32
4	16	32
8	16	32
12[b] /0	16	8
13/1	128	128
15/3	256	512
20/8	64	128
26/12. . .		

DETD CONSTRUCTION OF NYVAC RECOMBINANTS EXPRESSING **FLAVIVIRUS** PROTEINS

DETD This example describes the construction of NYVAC donor plasmids containing genes from Japanese **encephalitis virus** (JEV), **yellow fever virus** (YF) and **Dengue** type 1, the isolation of the corresponding NYVAC **Flavivirus** recombinants and the ability of vaccinia recombinants expressing portions of the genomes of JEV or YF to protect mice against. . .

DETD . . . and AccI fragment of JEV2 (Mason et al., 1991) containing the plasmid origin and JEV cDNA encoding the carboxy-terminal 40% **prM** and amino-terminal two thirds of **E** (nucleotides 602 to 2124), generating plasmid JEV20 containing JE sequences from the ATG of C through the SacI site (nucleotide 2124) found in the last third of **E**.

DETD . . . 1991) in which TTTTTGT nucleotides 1304 to 1310 were changed to TCTTTGT), containing JE sequences from the last third of **E** through the first two amino acids of NS2B (nucleotides 2124 to 4126), the plasmid

origin and vaccinia sequences, was ligated. . .

DETD . . . end] generated plasmid JEV25 which contains JE cDNA extending from the SacI site (nucleotide 2124) in the last third of **E** through the carboxy-terminus of **E**. The SacI-EagI fragment from JEV25 was ligated to the SacI-EagI fragment of JEV8 (containing JE cDNA encoding 15 aa C, **prM** and amino-terminal two thirds of **E** nucleotides 337 to 2124, the plasmid origin and vaccinia sequences) yielding plasmid JEV26. A unique SmaI site preceding the ATG. . .

DETD . . . fragment from JEV7 (Mason et al., 1991) yielded JEV29 (containing a SmaI site followed by JE cDNA encoding 30 aa **E**, NS1, NS2A nucleotides 2293 to 4126) and JEV30 (containing a SmaI site followed by JE cDNA encoding 30 aa **E**, NS1, NS2A, NS2B nucleotides 2293 to 4512).

DETD . . . Vitro Virus Infection and Radiolabeling. HeLa cell monolayers were prepared in 35 mm diameter dishes and infected with vaccinia viruses (**m**.o.i. of 2 pfu per cell) or JEV (**m**.o.i. of 5 pfu per cell) before radiolabeling. Cells were pulse labeled with medium containing ^{35} S-Met and chased for 6. . .

DETD Recombinant vP825 encoded the capsid protein, structural protein precursor **prM**, the structural glycoprotein **E**, the nonstructural glycoprotein NS1, and the nonstructural protein NS2A (McAda et al., 1987). Recombinant vP829 encoded the putative 15 aa **signal sequence** preceding the amino-terminus of **prM**, as well as **prM**, and **E** (McAda et al., 1987). Recombinant vP857 contained a cDNA encoding the 30 aa hydrophobic carboxy-terminus of **E**, followed by NS1 and NS2A. Recombinant vP864 contained a cDNA encoding the same proteins as vP857 with the addition of NS2B. In recombinants vP825 and vP829 a potential vaccinia virus early transcription termination signal in **E** (TTTTTGT; nucleotides 1304-1310) was modified to TCTTTGT without altering the aa sequence. This change was made in an attempt to increase the level of expression of **E** since this sequence has been shown to increase transcription termination in in vitro transcription assays (Yuen et al., 1987).

DETD **E** and **prM** Are Correctly Processed When Expressed By Recombinant Vaccinia Viruses. Pulse-chase experiments demonstrate that proteins identical in size to **E** were synthesized in cells infected with all recombinant vaccinia viruses containing the **E** gene (Table 16). In the case of cells infected with JEV, vP555 and vP829, an **E** protein that migrated slower in SDS-PAGE was also detected in the culture fluid harvested from the infected cells (Table 16). This extracellular form of **E** produced by JEV- and vP555-infected cells contained mature N-linked glycans (Mason, 1989; Mason et al., 1991), as confirmed for the extracellular forms of **E** produced by vP829-infected cells. Interestingly, vP825, which contained the C coding region in addition to **prM** and **E** specified the synthesis of **E** in a form that is not released into the extracellular fluid (Table 16). Immunoprecipitations prepared from radiolabeled recombinant vaccinia-infected cells using a MAb specific for **M** (and **prM**) revealed that **prM** was synthesized in cells infected with vP555, vP825, and vP829, and **M** was detected in the culture fluid of cells infected with vP555 or vP829 (Table 16).

DETD . . . (data not shown). This result indicated that vP829 infected cells produce extracellular particles similar to the empty vital envelopes containing **E** and **M** observed in the culture fluids harvested from vP555 infected cells (Table 16 and Mason et al., 1991).

DETD . . . To JEV Antigens. Pre-challenge sera pooled from selected animals in each group were tested for their ability to immunoprecipitate radiolabeled **E** and NS1. The results of these studies (Table 16) demonstrated that: (1) the magnitude of immune response induced to **E** was vP829>vP555>vP825, (2) all viruses encoding NS1 and NS2A induced antibodies to NS1, and (3) all immune responses were increased. . . sera collected from these animals (Table 17) confirmed the results of the immunoprecipitation analyses, showing that the immune response to **E** as demonstrated by RIP correlated well with these other serological tests (Table 17).

DETD TABLE 16

Characterization of proteins expressed by vaccinia recombinants expressing JEV proteins and their immune responses

	vP555	vP829	vP825	vP857	vP864
Proteins expressed[a]					
Intracellular	**prM,E** NS1	**prM,E**	**prM,E** NS1	NS1	NS1
secreted	**M,E**,NS1	**M,E**	none	NS1	NS1
Particle formation[b]	+	+	-	-	-
Immune response[c]					
single	**E**	**E**	NS1	NS1	NS1
double	**E**,NS1	**E**	**E**,NS1	NS1	NS1

[a] Radiolabelled cell lysates and culture fluids from vaccinia virus JEV recombinant infected cells were harvested and JEVspecific proteins immunoprecipitated using mAbs to **E**, **M** and NS1 proteins.

[b] Formation of extracellular particles with HA activity as described in the text.

[c] JEV proteins were. . .

DETD . . . isolated and ligated to a SacI (JEV nucleotide 2124) to EagI fragment of JEV25 (containing the remaining two thirds of **E**, translation stop and T5NT) generating JEV36. JEV36 was transfected into vP866 (NYVAC) infected cells to generate the vaccinia recombinant vP923. . .

DETD Plasmid YF0 containing YF cDNA encoding the carboxy-terminal 80% **prM**, **E** and amino-terminal 80% NS1 (nucleotides 537-3266) was derived by cloning an AvaI to NsiI fragment of YF cDNA (nucleotides 537-1659). . . and KpnI digested IBI25 (International Biotechnologies, Inc., New Haven, Conn.). Plasmid YF1 containing YF cDNA encoding C and amino-terminal 20% **prM** (nucleotides 119-536) was derived by cloning a RsaI to AvaI fragment of YF cDNA (nucleotides 166-536) and annealed oligonucleotides SP46. . . and YF nucleotides 119-165) into AvaI and HindIII digested IBI25. Plasmid YF3 containing YF cDNA encoding the carboxy-terminal 60% of **E** and amino-terminal 25% of NS1 was generated by cloning an ApaI to BamHI fragment of YF cDNA (nucleotides 1604-2725) into. . . cDNA (nucleotides 4339-4940) into SacI and XbaI digested IBI25. Plasmid YF13 containing YF cDNA encoding the carboxy-terminal 25% of C, **prM** and amino-terminal 40% of **E** was derived by cloning a BalI to ApaI fragment of YF cDNA (nucleotides 384-1603) into ApaI and SmaI digested IBI25.

DETD . . . in YF1 (TTTTTCT nucleotides 263-269 and TTTTTGT nucleotides 269-275) to (SEQ ID NO:122) TTCTTCTTCTTGT creating plasmid YF1B, (2) in the **E** gene in YF3 (nucleotides 1886-1893 TTTTTTGT to TTCTTTGT 189 aa from the carboxy-terminus and nucleotides 2429-2435 TTTTTGT to TTCTTGT 8. . . YF3C (nucleotides 1965-2725) was exchanged for the corresponding fragment of YF3B generating YF4 containing YF cDNA encoding the carboxy-terminal 60% **E** and amino-terminal 25% NS1 (nucleotides 1604-2725) with both mutagenized transcription termination signals. An ApaI to BamHI fragment from YF4 (nucleotides. . . 1604-2725) was substituted for the equivalent region in YF 0 creating plasmid YF6 containing YF cDNA encoding the carboxy-terminal 80% **prM**, **E** and amino-terminal 80% NS1 (nucleotides 537-3266) with both mutagenized transcription termination signals. Plasmid YF6 was digested with EcoRV within the. . .

DETD . . . described above was used (1) to insert XhoI and ClaI sites preceding the ATG 17 aa from the carboxy-terminus of **E** (nucleotides 2402-2404) in plasmid YF3C creating YF5, (2) to insert XhoI and ClaI sites preceding the ATG 19 aa from the carboxy-terminus of **prM** (nucleotides 917-919) in plasmid YF13 creating YF14, (3) to insert an XhoI site preceding the ATG 23 aa from the carboxy-terminus of **E** (nucleotides 2384-2386) in plasmid YF3C creating plasmid YF25, (4) and to insert an XhoI site and ATG (nucleotide 419) in. . .

DETD . . . YF5 (nucleotides 1604-2725) was exchanged for the corresponding

region of YF6 creating YF7 containing YF cDNA encoding the carboxy-terminal 80% **prM**, **E** and amino-terminal 80% NS1 (nucleotides 537-3266) with XhoI and ClaI sites at 2402 (17 aa from the carboxy-terminus of **E**) and a mutagenized transcription termination signal at 2429-2435 (8 aa from the carboxy-terminus of **E**). The ApaI to BamHI fragment from YF25 (nucleotides 1604-2725) was exchanged for the corresponding region of YF0 generating YF26 containing YF cDNA encoding the carboxy-terminal 80% **prM**, **E** and amino-terminal 80% NS1 (nucleotides 537-3266) with an XhoI site at nucleotide 2384 (23 aa from the carboxy-terminus of **E**) and mutagenized transcription termination signal at 2428-2435 (8 aa from the carboxy-terminus of **E**).

DETD . . . YF14 (nucleotides 537-1603) was substituted for the corresponding region in YF6 generating YF15 containing YF cDNA encoding the carboxy-terminal 80% **prM**, **E** and amino-terminal 80% NS1 (nucleotides 537-3266) with XhoI and ClaI sites at nucleotide 917 (19 aa from the carboxy-terminus of **prM**) and two mutagenized transcription termination signals. YF6 was digested within IBI25 with EcoRV and within YF at nucleotide 537 with. . .

DETD . . . from YF12 encoding carboxy-terminal 20% NS1, NS2A and NS2B (nucleotides 3267-4569), XhoI to KpnI fragment from YF15 encoding 19 aa **prM**, **E** and amino-terminal 80% NS1 (nucleotides 917-3266) and XhoI-SmaI digested pHES4 were ligated generating YF23. An XhoI to BamHI fragment from YF26 encoding 23 aa **E**, amino-terminal 25% NS1 (nucleotides.2384-2725) was ligated to an XhoI to BamHI fragment from YF23 (containing the carboxy-terminal 75% NS1,. . .

DETD XhoI-SmaI digested pHES4 was ligated to a purified XhoI to KpnI fragment from YF7 encoding 17 aa **E** and amino-terminal 80% NS1 (nucleotides 2402-3266) plus a KpnI to SmaI fragment from YF10 encoding the carboxy-terminal 20% NS1 and NS2A (nucleotides 3267-4180) creating YF18. An XhoI to BamHI fragment from YF2 encoding C, **prM**, **E** and amino-terminal 25% NS1 (nucleotides 119-2725) was ligated to a XhoI to BamHI fragment of YF18 (containing the carboxy-terminal 75%. . . the origin of replication and vaccinia sequences) generating YF20. A XhoI to BamHI fragment from YF46 encoding 21 aa C, **prM**, **E** and amino-terminal 25% NS1 (nucleotides 419-2725) was ligated to the XhoI to BamHI fragment from YF18 generating YF47. Oligonucleotide SP46. . .

DETD Recombinant vP725 encoded the putative 17-aa **signal sequence** preceding the N terminus of the nonstructural protein NS1 and the nonstructural proteins NS1 and NS2A (Rice et al., 1985). Recombinant vP729 encoded the putative 19-aa **signal sequence** preceding the N terminus of **E**, **E**, NS1, NS2A and NS2B (Rice et al., 1985). Recombinant vP764 encoded C, **prM**, **E**, NS1, NS2A and NS2B (Rice et al., 1985). Recombinant vP766 encoded C, **prM**, **E**, NS1 and NS2A (Rice et al., 1985). Recombinant vP869 encoded the putative 21-aa **signal sequence** preceding the N terminus of the **prM** structural protein precursor as well as **prM**, **E**, NS1 and NS2A (Rice et al., 1985).

DETD . . . NYVAC Donor Plasmid. A XhoI to SmaI fragment from YF47 (nucleotides 419-4180) containing YF cDNA encoding 21 amino acids C, **prM**, **E**, NS1, NS2A (with nucleotide 2962 missing in NS1) was ligated to XhoI-SmaI digested SPHA-H6 (HA region donor plasmid) generating YF48.. . . (nucleotide 3262) and a 6700 bp fragment isolated (containing the plasmid origin of replication, vaccinia sequences, 21 amino acids C, **prM**, **E**, amino-terminal 3.5% NS1, carboxy-terminal 23% NS1, NS2A) and ligated to a SacI-Asp718 fragment from YF18 (containing the remainder of NS1. . . site in YF51 were removed using oligonucleotide-directed double-strand break mutagenesis (Mandecki, 1986) creating plasmid YF50 encoding YF21 amino acids C, **prM**, **E**, NS1, NS2A in the HA locus donor plasmid. Donor plasmid YF50 was transfected into vP866 (NYVAC) infected cells to generate. . .

DETD . . . double-strand break mutagenesis creating YF49. Oligonucleotide-directed mutagenesis (Kunkel, 1985) was used to insert a SmaI site at the carboxy-terminus of **E** (nucleotide 2452) in YF4 creating YF16. An ApaI-SmaI fragment of YF49 (containing the plasmid origin of replication, vaccinia sequences and YF cDNA encoding 21 amino acid C, **prM**, and amino-terminal 43% **E**) was ligated to an ApaI-SmaI fragment from YF16 (nucleotides 1604-2452 containing the

truncated hepatitis C virus core protein.

28. The recombinant DNA molecule of claim 27 wherein said fusion protein consists of the hepatitis B virus S gene product linked to a truncated hepatitis C virus core protein.

29. The recombinant DNA molecule of claim 27 wherein said fusion protein consists of a fragment of the hepatitis B virus pre S2 gene product linked to the hepatitis B virus S gene product linked to a truncated hepatitis C virus core protein.

30. The recombinant DNA molecule of claim 27 wherein said fusion protein consists of the hepatitis B virus pre S2 gene product linked to the hepatitis B virus S gene product linked to a truncated hepatitis C virus core protein.

31. A pharmaceutical composition comprising: a) a recombinant DNA molecule of claim 1; wherein said nucleotide sequence is operably linked to regulatory elements functional in human cells; and b) a pharmaceutically acceptable carrier or diluent.

32. The pharmaceutical composition of claim 31 wherein said regulatory elements functional in human cells comprise a **cytomegalovirus** promoter, a Rous Sarcoma Virus enhancer, a polyadenylation sequence.

33. The pharmaceutical composition of claim 32 further comprising the 5' UTR of hepatitis C virus.

34. A method of immunizing an individual susceptible to or infected by hepatitis C virus for producing antibodies comprising the step of: administering to said individual the pharmaceutical composition of claim 31 in an amount effective to induce an immune response, wherein antibodies are produced.

35. The method of claim 34 wherein bupivacaine is administered to said individual at the site of administration of the pharmaceutical composition.

36. A pharmaceutical composition comprising: a) the recombinant DNA molecule of claim 5, wherein said nucleotide sequence is operably linked to regulatory elements functional in human cells; and b) a pharmaceutically acceptable carrier or diluent.

37. The pharmaceutical composition of claim 36 wherein said regulatory elements functional in human cells comprise a **cytomegalovirus** promoter, a Rous Sarcoma Virus enhancer, a polyadenylation sequence.

38. The pharmaceutical composition of claim 37 further comprising the 5' UTR of hepatitis C virus.

39. A method of immunizing an individual susceptible to or infected by hepatitis C virus for producing antibodies comprising the step of: administering to said individual the pharmaceutical composition of claim 36 in an amount effective to induce an immune response, wherein antibodies are produced.

40. The method of claim 39 wherein bupivacaine is administered to said individual at the site of administration of the pharmaceutical composition.

41. A pharmaceutical composition comprising: a) the recombinant DNA molecule of claim 9, wherein said nucleotide sequence is operably linked to regulatory elements functional in human cells; and b) a pharmaceutically acceptable carrier or diluent.

42. The pharmaceutical composition of claim 41 wherein said regulatory

elements functional in human cells comprise a **cytomegalovirus** promoter, a Rous Sarcoma Virus enhancer, a polyadenylation sequence.

43. The pharmaceutical composition of claim 42 further comprising the 5' UTR of hepatitis C virus.

44. A method of immunizing an individual susceptible to or infected by hepatitis C virus for producing antibodies comprising the step of: administering to said individual the pharmaceutical composition of claim 41 in an amount effective to induce an immune response, wherein antibodies are produced.

45. The method of claim 44 wherein bupivacaine is administered to said individual at the site of administration of the pharmaceutical composition.

46. A pharmaceutical composition comprising: a) the recombinant DNA molecule of claim 13, wherein said nucleotide sequence is operably linked to regulatory elements functional in human cells; and b) a pharmaceutically acceptable carrier or diluent.

47. The pharmaceutical composition of claim 46 wherein said regulatory elements functional in human cells comprise a **cytomegalovirus** promoter, a Rous Sarcoma Virus enhancer, a polyadenylation sequence.

48. The pharmaceutical composition of claim 47 further comprising the 5' UTR of hepatitis C virus.

49. A method of immunizing an individual susceptible to or infected by hepatitis C virus for producing antibodies comprising the step of: administering to said individual the pharmaceutical composition of claim 46 in an amount effective to induce an immune response, wherein antibodies are produced.

50. The method of claim 49 wherein bupivacaine is administered to said individual at the site of administration of the pharmaceutical composition.

51. A pharmaceutical composition comprising: a) the recombinant DNA molecule of claim 17, wherein said nucleotide sequence is operably linked to regulatory elements functional in human cells; and b) a pharmaceutically acceptable carrier or diluent.

52. The pharmaceutical composition of claim 51 wherein said regulatory elements functional in human cells comprise a **cytomegalovirus** promoter, a Rous Sarcoma Virus enhancer, a polyadenylation sequence.

53. The pharmaceutical composition of claim 52 further comprising the 5' UTR of hepatitis C virus.

54. A method of immunizing an individual susceptible to or infected by hepatitis C virus for producing antibodies comprising the step of: administering to said individual the pharmaceutical composition of claim 51 in an amount effective to induce an immune response, wherein antibodies are produced.

55. The method of claim 54 wherein bupivacaine is administered to said individual at the site of administration of the pharmaceutical composition.

56. A pharmaceutical composition comprising: a) the recombinant DNA molecule of claim 21, wherein said nucleotide sequence is operably linked to regulatory elements functional in human cells; and b) a pharmaceutically acceptable carrier or diluent.

57. The pharmaceutical composition of claim 56 wherein said regulatory

elements functional in human cells comprise a cytomegalovirus promoter, a Rous Sarcoma Virus enhancer, a polyadenylation sequence.

58. The pharmaceutical composition of claim 57 further comprising the 5' UTR of hepatitis C virus.

59. A method of immunizing an individual susceptible to or infected by hepatitis C virus for producing antibodies comprising the step of: administering to said individual the pharmaceutical composition of claim 56 in an amount effective to induce an immune response, wherein antibodies are produced.

60. The method of claim 59 wherein bupivacaine is administered to said individual at the site of administration of the pharmaceutical composition.

L23 ANSWER 6 OF 10 USPATFULL on STN
2000:7195 Method for stimulating an immune response utilizing recombinant alphavirus particles.
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Polo, John M., San Diego, CA, United States
Chang, Steven M.W., San Diego, CA, United States
Jolly, Douglas J., Leucadia, CA, United States
Chiron Corporation, Emeryville, CA, United States (U.S. corporation)
US 6015694 20000118
APPLICATION: US 1997-931869 19970916 (8) <--
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention provides compositions and methods for utilizing recombinant alphavirus vectors. Also disclosed are compositions and methods for making and utilizing eukaryotic layered vector initiation systems.
CLM What is claimed is:
1. A method of stimulating in an animal an immune response to an antigen, comprising infecting susceptible animal target cells with recombinant alphavirus particles comprising a vector which directs the expression of at least one antigen or modified form thereof in target cells infected with the alphavirus, wherein said antigen or modified form thereof stimulates an immune response within an animal, and wherein said recombinant alphavirus particles are obtained from an alphavirus packaging cell comprising a stably transformed expression cassette which expresses an alphavirus structural protein, which, after introduction of an alphavirus vector construct, produces recombinant alphavirus particles.

2. The method according to claim 1 wherein said antigen is a viral antigen.

3. The method according to claim 2 wherein said viral antigen is obtained from a virus selected from the group consisting of influenza virus, respiratory syncytial virus, HPV, HBV, HIV, HSV, FeLV, FIV, HTLV-1, HTLV-2, and **CMV**.

4. The method according to claim 2 wherein said viral antigen is obtained from a hepatitis C virus.

5. The method acording to claim 1 wherein said antigen is a tumor antigen.

6. The method according to claim 1 wherein said antigen is obtained from a bacteria, parasite or fungus.

7. The method according to claim 1, wherein said alphavirus vector construct is introduced into said packaging cell by transfecting a eukaryotic layered vector initiation system or an alphavirus vector

construct RNA into said packaging cell.

8. The method according to claim 1, wherein said alphavirus vector construct is introduced into said packaging cell by infecting said packaging cell with a recombinant alphavirus particle.

9. A method of stimulating in an animal an immune response to an antigen, comprising infecting susceptible animal target cells with recombinant alphavirus particles which direct the expression of at least one antigen ormodified form thereof in target cells infected with the alphavirus, wherein said antigen or modified form thereof stimulates an immune response within an animal, and wherein said recombinant alphavirus particles are free from recombinant alphavirus particles that can initiate a productive infection that yields infective alphavirus particles.

10. A method according to any one of claims 1 or 9 wherein the target cells are infected within said animal.

11. A method according to any one of claims 1 or 9 wherein the expressed antigen elicits an immune response selected from the group consisting of a cell-mediated immune response, a HLA class I-restricted immune response, and a HLA Class II-restricted immune response.

L23 ANSWER 7 OF 10 USPATFULL on STN
1999:141912 Compositions and methods for delivery of genetic material.
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The Trustees of The University of Pennsylvania, Philadelphia, PA, United States (U.S. corporation)The Wistar Institute, Philadelphia, PA, United States (U.S. corporation)
US 5981505 19991109
WO 9416737 19940804
APPLICATION: US 1997-979385 19971126 (8) <--
WO 1994-US899 19940126 19950828 PCT 371 date 19950828 PCT 102(e) date<--
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Methods of inducing genetic material into cells of an individual and compositions and kits for practicing the same are disclosed. The methods comprise the steps of contacting cells of an individual with a polynucleotide function enhancer and administering to the cells, a nucleic acid molecule that is free of retroviral particles. The nucleic acid molecule comprises a nucleotide sequence that encodes a protein that comprises at least one epitope that is identical or substantially similar to an epitope of a pathogen antigen or an antigen associated with a hyperproliferative or autoimmune disease, a protein otherwise missing from the individual due to a missing, non-functional or partially functioning gene, or a protein that produces a therapeutic effect on an individual. Methods of prophylactically and therapeutically immunizing an individual against HIV are disclosed. Pharmaceutical compositions and kits for practicing methods of the present invention are disclosed.
CLM What is claimed is:
1. A pharmaceutical composition comprising: a) a polynucleotide function enhancer; and b) a DNA molecule that comprises a DNA sequence that encodes an antigen; wherein said polynucleotide function enhancer is selected from the group consisting of bupivacaine and tetracaine and said DNA sequence operatively linked to regulatory sequences which control the expression of said DNA sequence.

2. The pharmaceutical composition of claim 1 wherein said composition comprises bupivacaine.

3. The pharmaceutical composition of claim 1 wherein said composition

comprises tetracaine.

4. The pharmaceutical composition of claim 1 wherein said DNA molecule is a plasmid.

5. The pharmaceutical composition of claim 1 wherein said DNA sequence encodes a variable region of a T cell receptor.

6. The pharmaceutical composition of claim 1 wherein said DNA sequence encodes a pathogen antigen.

7. The pharmaceutical composition of claim 6 wherein said DNA sequence encodes an antigen from an intracellular pathogen.

8. The pharmaceutical composition of claim 7 wherein said antigen is a viral antigen.

9. The pharmaceutical composition of claim 7 wherein said pathogen is a virus selected from the group consisting of: human immunodeficiency virus, HIV; human T cell leukemia virus, HTLV; influenza virus; hepatitis A virus; hepatitis B virus; hepatitis C virus; human papilloma virus, HPV; Herpes simplex 1 virus, HSV1; Herpes simplex 2 virus, HSV2; **Cytomegalovirus, CMV**; Epstein-Barr virus, EBR; rhinovirus; and, coronavirus.

10. The pharmaceutical composition of claim 9 wherein said pathogen is Herpes simplex 2 virus, HSV2.

11. The pharmaceutical composition of claim 9 wherein said pathogen is Hepatitis B virus, HBV.

12. The pharmaceutical composition of claim 9 wherein said pathogen is human T cell leukemia virus, HTLV.

13. The pharmaceutical composition of claim 9 wherein said pathogen is a human immunodeficiency virus.

14. The pharmaceutical composition of claim 1 wherein said DNA sequence encodes a hyperproliferative disease associated protein.

15. The pharmaceutical composition of claim 14 wherein said hyperproliferative disease is cancer.

16. The pharmaceutical composition of claim 14 wherein said hyperproliferative disease is a lymphoma.

17. The pharmaceutical composition of claim 14 wherein said hyperproliferative disease is a melanoma.

18. A method of immunizing an individual comprising the steps of: injecting into tissue of said individual at a site on said individual's body, a DNA molecule and a polynucleotide function enhancer, said DNA molecule comprising a DNA sequence that encodes an antigen, said DNA sequence operatively linked to regulatory sequences which control the expression of said DNA sequence, said polynucleotide function enhancer selected from the group consisting of bupivacaine and tetracaine; wherein said DNA molecule is taken up by cells in said tissue, said DNA sequence is expressed in said cells and an immune response is generated against said antigen.

19. The method of claim 18 wherein said tissue includes skin and skeletal muscle.

20. The method of claim 18 wherein said tissue is skin.

21. The method of claim 18 wherein said tissue is muscle.

22. The method of claim 18 wherein said tissue is skeletal muscle.

23. The method of claim 18 wherein said polynucleotide function enhancer is bupivacaine.

24. The method of claim 18 wherein said polynucleotide function enhancer is tetracaine.

25. The method of claim 18 wherein said DNA molecule is a plasmid.

26. The method of claim 18 wherein said immune response generated against said antigen is an immune response against a pathogen antigen.

27. The method of claim 26 wherein said pathogen is an intracellular pathogen.

28. The method of claim 27 wherein said intracellular pathogen is a virus.

29. The method of claim 26 wherein said pathogen is a virus selected from the group consisting of: human immunodeficiency virus, HIV; human T cell leukemia virus, HTLV; influenza virus; hepatitis a virus; hepatitis B virus; hepatitis C virus; human papilloma virus, HPV; Herpes simplex 1 virus, HSV1; Herpes simplex 2 virus, HSV2; **Cytomegalovirus, CMV;** Epstein-Barr virus, EBR; rhinovirus; and, coronavirus.

30. The method of claim 18 wherein said immune response generated against said antigen provides a protective immune response against a pathogen and said individual is immunized against said pathogen.

31. The method of claim 30 wherein said tissue is skin.

32. The method of claim 30 wherein said tissue is skeletal muscle.

33. The method of claim 30 wherein said polynucleotide function enhancer is bupivacaine.

34. The method of claim 30 wherein said polynucleotide function enhancer is tetracaine.

35. The method of claim 30 wherein said DNA molecule is a plasmid.

36. The method of claim 30 wherein said antigen is a pathogen antigen.

37. The method of claim 30 wherein said pathogen is an intracellular pathogen.

38. The method of claim 37 wherein said intracellular pathogen is a virus.

39. The method of claim 38 wherein said virus is selected from the group consisting of: human immunodeficiency virus, HIV; human T cell leukemia virus, HTLV; influenza virus; hepatitis A virus; hepatitis B virus; hepatitis C virus; human papilloma virus, HPV; Herpes simplex 1 virus, HSV1; Herpes simplex 2 virus, HSV2; **Cytomegalovirus, CMV;** Epstein-Barr virus, EBR; rhinovirus; and, coronavirus.

40. The method of claim 39 wherein said virus is Herpes simplex 2 virus, HSV2.

41. The method of claim 39 wherein said virus is Hepatitis B virus, HBV.

42. The method of claim 39 wherein said virus is human T cell leukemia virus, HTLV.

43. The method of claim 18 wherein said immune response generated against said antigen provides a therapeutic immune response against a pathogen in an individual who is infected with said pathogen.

44. The method of claim 43 wherein said tissue is skin.

45. The method of claim 43 wherein said tissue is skeletal muscle.

46. The method of claim 43 wherein said polynucleotide function enhancer is bupivacaine.

47. The method of claim 43 wherein said polynucleotide function enhancer is tetracaine.

48. The method of claim 43 wherein said DNA molecule is a plasmid.

49. The method of claim 43 wherein said pathogen is an intracellular pathogen.

50. The method of claim 43 wherein said pathogen is a virus.

51. The method of claim 18 wherein said immune response generated against said antigen is an immune response against a hyperproliferative disease-associated protein.

52. The method of claim 51 wherein said tissue is skin.

53. The method of claim 51 wherein said tissue is skeletal muscle.

54. The method of claim 51 wherein said polynucleotide function enhancer is bupivacaine.

55. The method of claim 51 wherein said polynucleotide function enhancer is tetracaine.

56. The method of claim 51 wherein said DNA molecule is a plasmid.

57. The method of claim 51 wherein said hyperproliferative disease-associated protein is selected from the group consisting of: protein products of oncogenes myb, myc, fyn, ras, src, neu and trk; protein products of translocation gene bcr/abl; P53; variable regions of antibodies made by B cell lymphomas; and variable regions of T cell receptors of T cell lymphomas.

58. The method of claim 51 wherein said immune response generated against said antigen is a therapeutically effective immune response against a hyperproliferative disease-associated protein in an individual who has a hyperproliferative disease.

59. The method of claim 58 wherein said hyperproliferative disease is cancer.

60. The method of claim 58 wherein said hyperproliferative disease is a melanoma.

61. The method of claim 58 wherein said hyperproliferative disease is a lymphoma.

62. The method of claim 18 wherein said immune response generated against said antigen is an immune response against an autoimmune disease-associated protein.

63. The method of claim 62 wherein said tissue is skin.

64. The method of claim 62 wherein said tissue is skeletal muscle.

65. The method of claim 62 wherein said polynucleotide function enhancer is bupivacaine.

66. The method of claim 62 wherein said polynucleotide function enhancer is tetracaine.

67. The method of claim 62 wherein said DNA molecule is a plasmid.

68. The method of claim 62 wherein said autoimmune disease associated-protein is selected from the group consisting of: variable regions of antibodies involved in B cell mediated autoimmune disease; and variable regions of T cell receptors involved in T cell mediated autoimmune disease.

69. A method of introducing DNA molecules into cells of an individual comprising the step of: injecting into tissue of said individual at a site on said individual's body, DNA molecules and a polynucleotide function enhancer selected from the group consisting of bupivacaine and tetracaine, wherein said DNA molecules are taken up by cells in said tissue.

70. The method of claim 69 wherein said DNA molecule comprises a DNA sequence that encodes an protein, said DNA sequence operatively linked to regulatory sequences which control the expression of said DNA sequence.

71. The method of claim 69 wherein said tissue is skin.

72. The method of claim 69 wherein said tissue is skeletal muscle.

73. The method of claim 69 wherein said polynucleotide function enhancer is bupivacaine.

74. The method of claim 69 wherein said polynucleotide function enhancer is tetracaine.

75. The method of claim 69 wherein said DNA molecule is a plasmid.

L23 ANSWER 8 OF 10 USPATFULL on STN
1999:121330 Compositions and methods for delivery of genetic material.
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Apollon, Inc., Malvern, PA, United States (U.S. corporation)The Trustees Of The University of Pennsylvania, Philadelphia, PA, United States (U.S. corporation)
US 5962428 19991005
WO 9526718 19951012
APPLICATION: US 1996-704701 19960916 (8) <--
WO 1995-US4071 19950330 19960916 PCT 371 date 19960916 PCT 102(e) date<--
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Methods of introducing genetic material into cells of an individual and compositions and kits for practicing the same are disclosed. The methods comprise the steps of contacting cells of an individual with a genetic vaccine facilitator and administering to the cells a nucleic acid molecule that is free of retroviral particles. The nucleic acid molecule comprises a nucleotide sequence that encodes a protein that comprises at least one epitope that is identical or substantially similar to an epitope of a pathogen antigen or an antigen associated with a hyperproliferative or autoimmune disease, a protein otherwise missing from the individual due to a missing, non-functional or partially functioning gene, or a protein that produces a therapeutic effect on an individual. Methods of prophylactically and therapeutically immunizing an individual against HIV are disclosed. Pharmaceutical compositions and

kits for practicing methods of the present invention are disclosed.

CLM What is claimed is:

1. A method of generating an immune response in an individual against an antigen comprising administering in vivo to muscle or skin of said individual's body a genetic vaccine facilitator selected from the group consisting of: anionic lipids; saponins; lectins; estrogenic compounds; hydroxylated lower alkyls; dimethyl sulfoxide; and urea; and, a DNA molecule that is dissociated from an infectious agent and comprises a DNA sequence that encodes said antigen, said DNA sequence operatively linked to regulatory sequences which control the expression of said DNA sequence; wherein said DNA molecule is taken up by cells, said DNA sequence is expressed in said cells and an immune response is generated against said antigen.

2. The method of claim 1 wherein said genetic vaccine facilitator is an anionic lipid selected from the group consisting of: salts of lauric and oleic acids, lauric and oleic acids, acid esters of lauryl and cetyl alcohol, and sulfonates.

3. The method of claim 1 wherein said genetic vaccine facilitator is an anionic lipid selected from the group consisting of: sodium lauryl sulfate and oleic acid.

4. The method of claim 1 wherein said genetic vaccine facilitator is a saponin selected from the group consisting of: saponarin, sarmentocymarin and sapogenins.

5. The method of claim 1 wherein said genetic vaccine facilitator is a saponin selected from the group consisting of: sarmentogenin, sarsasapogenin and sarverogenin.

6. The method of claim 1 wherein said genetic vaccine facilitator is a lectin selected from the group consisting of: concanavalin A, abrin, soybean agglutinin and wheat germ agglutinin.

7. The method of claim 1 wherein said genetic vaccine facilitator is concanavalin A.

8. The method of claim 1 wherein said genetic vaccine facilitator is β-estradiol.

9. The method of claim 1 wherein said genetic vaccine facilitator is selected from the group consisting of: ethanol, n-propanol, isopropanol and n-butanol.

10. The method of claim 1 wherein said genetic vaccine facilitator is dimethyl sulfoxide.

11. The method of claim 1 wherein said genetic vaccine facilitator is urea.

12. A method of generating an immune response in an individual against a pathogen comprising administering in vivo to muscle or skin of said individual's body a genetic vaccine facilitator selected from the group consisting of: anionic lipids; saponins; lectins; estrogenic compounds; hydroxylated lower alkyls; dimethyl sulfoxide; and urea; and, a DNA molecule that is dissociated from an infectious agent and comprises a DNA sequence that encodes a protein which comprises at least one epitope that is identical or substantially similar to an epitope of a pathogen antigen, said nucleotide sequence being operably linked to regulatory sequences; wherein said DNA molecule is taken up by cells, said DNA sequence is expressed in said cells and an immune response is generated against said antigen.

13. The method of claim 12 wherein said genetic vaccine facilitator is selected from the group consisting of: sodium lauryl sulfate; oleic

acid; saponarin; sarmentocymarin; sapogenins; sarmentogenin; sarsasapogenin; sarverogenin; concanavalin A; β-estradiol; ethanol; dimethyl sulfoxide; and urea.

14. The method of claim 12 wherein said DNA molecule is a plasmid.

15. The method of claim 12 wherein said protein is a pathogen antigen or a fragment thereof which is antigenic.

16. The method of claim 12 wherein said DNA molecule is administered intramuscularly.

17. The method of claim 12 wherein said pathogen is a virus selected from the group consisting of: human immunodeficiency virus, HIV; human T cell leukemia virus, HTLV; influenza virus; hepatitis A virus, HAV; hepatitis B virus, HBV; hepatitis C virus, HCV; human papilloma virus, HPV; Herpes simplex 1 virus, HSV1; Herpes simplex 2 virus, HSV2; **Cytomegalovirus, CMV**; Epstein-Barr virus, EBV; rhinovirus; and, coronavirus.

18. The method of claim 12 wherein at least two or more different nucleic acid molecules are administered to different cells of an individual; said different nucleic acid molecules each comprising DNA sequences encoding one or more pathogen antigens of the same pathogen.

19. The method of claim 12 wherein said genetic vaccine facilitator and said DNA molecule are administered simultaneously.

20. A method of generating an immune response in an individual against a disease comprising administering in vivo to muscle or skin of said individual's body a genetic vaccine facilitator selected from the group consisting of: anionic lipids; saponins; lectins; estrogenic compounds; hydroxylated lower alkyls; dimethyl sulfoxide; and urea; and, a DNA molecule that is dissociated from an infectious agent and comprises a DNA sequence that encodes a protein which comprises an epitope identical or substantially similar to an epitope of a protein associated with said disease operatively linked to regulatory sequences; wherein said DNA molecule is taken up by cells, said DNA sequence is expressed in said cells and an immune response is generated against said target protein.

21. The method of claim 20 wherein said genetic vaccine facilitator is selected from the group consisting of: sodium lauryl sulfate; oleic acid; saponarin; sarmentocymarin; sapogenins; sarmentogenin; sarsasapogenin; sarverogenin; concanavalin A; β-estradiol; ethanol; dimethyl sulfoxide; and urea.

22. The method of claim 20 wherein said disease is characterized by hyperproliferating cells.

23. The method of claim 20 wherein said disease is an autoimmune disease.

24. The method of claim 20 wherein said DNA molecule is a plasmid.

25. The method of claim 20 wherein said DNA molecule is administered intramuscularly.

26. The method of claim 20 wherein said DNA molecule comprises a DNA sequence that encodes a protein selected from the group consisting of: protein products of oncogenes myb, myc, fyn, ras, sarc, neu and trk; protein products of translocation gene bcl/abl; p53; EGRF; variable regions of antibodies made by B cell lymphomas; and variable regions of T cell receptors of T cell lymphomas.

27. The method of claim 20 wherein said protein is selected from the group consisting of: variable regions of antibodies involved in B cell

mediated autoimmune disease, and variable regions of T cell receptors involved in T cell mediated autoimmune disease.

28. A pharmaceutical composition comprising: i) a DNA molecule that is dissociated from an infectious agent and comprises a DNA sequence which encodes a protein wherein said DNA sequence is operably linked to regulatory sequences required for expression in a mammal and said protein is selected from the group consisting of: proteins which comprise at least one epitope that is identical or substantially similar to an epitope of a pathogen antigen; proteins which comprise an epitope identical or substantially similar to an epitope of a protein associated with hyperproliferating cells; and proteins which comprise an epitope identical or substantially similar to an epitope of a protein associated with an autoimmune disease; and ii) a genetic vaccine facilitator selected from the group consisting of anionic lipids; saponins; lectins; estrogenic compounds; hydroxylated lower alkyls; dimethyl sulfoxide; and urea.

29. The pharmaceutical composition of claim 28 wherein said genetic vaccine facilitator is selected from the group consisting of: sodium lauryl sulfate; oleic acid; saponarin; sarmentocymarin; sapogenins; sarmentogenin; sarsasapogenin; sarverogenin; concanavalin A; β-estradiol; ethanol; dimethyl sulfoxide; and urea.

30. A pharmaceutical kit comprising: i) a container that comprises a DNA molecule that is dissociated from an infectious agent and comprises a DNA sequence which encodes a protein wherein said DNA sequence is operably linked to regulatory sequences required for expression in a mammal and said protein is selected from the group consisting of: proteins which comprise at least one epitope that is identical or substantially similar to an epitope of a pathogen antigen; proteins which comprise an epitope identical or substantially similar to an epitope of a protein associated with hyperproliferating cells; and proteins which comprise an epitope identical or substantially similar to an epitope of a protein associated with an autoimmune disease; and ii) a container that comprises a genetic vaccine facilitator selected from the group consisting of: anionic lipids; saponins; lectins; estrogenic compounds; hydroxylated lower alkyls; dimethyl sulfoxide; and urea.

31. The pharmaceutical kit of claim 30 wherein said genetic vaccine facilitator is selected from the group consisting of: sodium lauryl sulfate; oleic acid; saponarin; sarmentocymarin; sapogenins; sarmentogenin; sarsasapogenin; sarverogenin; concanavalin A; β-estradiol; ethanol; dimethyl sulfoxide; and urea.

32. A method of delivering a protein into cells of an individual in vivo comprising administering to muscle or skin of said individual's body a genetic vaccine facilitator selected from the group consisting of: anionic lipids; saponins; lectins; estrogenic compounds; hydroxylated lower alkyls; dimethyl sulfoxide; and urea; and, a DNA molecule that is dissociated from an infectious agent and comprises a DNA sequence that encodes said protein, said DNA sequence operatively linked to regulatory sequences which control the expression of said DNA sequence; wherein said DNA molecule is taken up by cells, said DNA sequence is expressed in said cells producing said protein in said cells.

33. The method of claim 30 wherein said genetic vaccine facilitator is an anionic lipid selected from the group consisting of: salts of lauric and oleic acids, lauric and oleic acids, acid esters of lauryl and cetyl alcohol, and sulfonates.

34. The method of claim 30 wherein said genetic vaccine facilitator is an anionic lipid selected from the group consisting of: sodium lauryl sulfate and oleic acid.

35. The method of claim 30 wherein said genetic vaccine facilitator is a

saponin selected from the group consisting of: saponarin, sarmentocymarin and sapogenins.

36. The method of claim 30 wherein said genetic vaccine facilitator is a saponin selected from the group consisting of: sarmentogenin, sarsasapogenin and sarverogenin.

37. The method of claim 30 wherein said genetic vaccine facilitator is a lectin selected from the group consisting of: concanavalin A, abrin, soybean agglutinin and wheat germ agglutinin.

38. The method of claim 30 wherein said genetic vaccine facilitator is concanavalin A.

39. The method of claim 30 wherein said genetic vaccine facilitator is β-estradiol.

40. The method of claim 30 wherein said genetic vaccine facilitator is selected from the group consisting of: ethanol, n-propanol, isopropanol and n-butanol.

41. The method of claim 30 wherein said genetic vaccine facilitator is dimethyl sulfoxide.

42. The method of claim 30 wherein said genetic vaccine facilitator is urea.

L23 ANSWER 9 OF 10 USPATFULL on STN
1998:122388 Genetic immunization.
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The Trustees of the University of Pennsylvania, Philadelphia, PA, United States (U.S. corporation)The Wistar Institute, Philadelphia, PA, United States (U.S. corporation)
US 5817637 19981006
APPLICATION: US 1997-783818 19970113 (8) <--
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Methods of prophylactic and therapeutic immunization of an individual against pathogen infection, diseases associated with hyperproliferative cells and autoimmune diseases are disclosed. The methods comprise the steps of administering to cells of an individual, a nucleic acid molecule that comprises a nucleotide sequence that encodes a protein which comprises at least one epitope that is identical or substantially similar to an epitope of a pathogen antigen, a hyperproliferative cell associated protein or a protein associated with autoimmune disease respectively. In each case, nucleotide sequence is operably linked to regulatory sequences to enable expression in the cells. The nucleic acid molecule is free of viral particles and capable of being expressed in said cells. The cells may be contacted cells with a cell stimulating agent. Methods of prophylactically and therapeutically immunizing an individual against HIV are disclosed. Pharmaceutical compositions and kits for practicing methods of the present invention are disclosed.
CLM What is claimed is:
1. An pharmaceutical immunizing kit comprising: a) a first inoculant comprising: i) a pharmaceutically acceptable carrier or diluent; and, ii) a first nucleic acid molecule comprising a nucleotide sequence that encodes at least one HIV protein operatively linked to regulatory sequences; wherein said nucleotide sequence is capable of being expressed in human cells; b) a second inoculant comprising: i) a pharmaceutically acceptable carrier or diluent; and, ii) a second nucleic acid molecule comprising a nucleotide sequence that encodes at least one HIV protein operatively linked to regulatory sequences; wherein said nucleotide sequence is capable of being expressed in human

cells, wherein said first nucleic acid molecule is not identical to said second nucleic acid molecule and, taken together, said first nucleic acid molecule and said second nucleic acid molecule encode HIV proteins gag, pol and env; and c) a third inoculant comprising bupivacaine.

2. A pharmaceutical composition comprising: a) a compound selected from the group consisting of: bupivacaine, mepivacaine, lidocaine, procaine, carbocaine and methyl bupivacaine; and b) a DNA molecule that comprises a DNA sequence that encodes an antigen; wherein said DNA sequence operatively linked to regulatory sequences which control the expression of said DNA sequence.

3. The pharmaceutical composition of claim 2 wherein said composition comprises bupivacaine.

4. The pharmaceutical composition of claim 2 wherein said DNA molecule is a plasmid.

5. The pharmaceutical composition of claim 2 wherein said DNA sequence encodes a variable region of a T cell receptor.

6. The pharmaceutical composition of claim 2 wherein said DNA sequence encodes a pathogen antigen.

7. The pharmaceutical composition of claim 6 wherein said DNA sequence encodes an antigen from an intracellular pathogen.

8. The pharmaceutical composition of claim 7 wherein said antigen is a viral antigen.

9. The pharmaceutical composition of claim 8 wherein said pathogen is a virus selected from the group consisting of: human immunodeficiency virus, HIV; human T cell leukemia virus, HTLV; influenza virus; hepatitis A virus; hepatitis B virus; hepatitis C virus; human papilloma virus, HPV; Herpes simplex 1 virus, HSV1; Herpes simplex 2 virus, HSV2; **Cytomegalovirus, CMV**; Epstein-Barr virus, EBR; rhinovirus; and, coronavirus.

10. The pharmaceutical composition of claim 9 wherein said pathogen is Herpes simplex 2 virus, HSV2.

11. The pharmaceutical composition of claim 9 wherein said pathogen is Hepatitis B virus, HBV.

12. The pharmaceutical composition of claim 9 wherein said pathogen is human T cell leukemia virus, HTLV.

13. The pharmaceutical composition of claim 9 wherein said pathogen is a human immunodeficiency virus.

14. The pharmaceutical composition of claim 2 wherein said DNA sequence encodes a hyperproliferative disease associated protein.

15. The pharmaceutical composition of claim 13 wherein said hyperproliferative disease is cancer.

16. The pharmaceutical composition of claim 14 wherein said hyperproliferative disease is a lymphoma.

17. The pharmaceutical composition of claim 14 wherein said hyperproliferative disease is a melanoma.

18. a method of immunizing an individual against an antigen comprising administering to tissue of said individual's body, a) a compound selected from the group consisting of bupivacaine, mepivacaine, lidocaine, procaine, carbocaine and methyl bupivacaine, and b) a DNA

molecule that comprises a DNA sequence that encodes said antigen, said DNA sequence operatively linked to regulatory sequences which control the expression of said DNA sequence; wherein said DNA molecule is taken up by cells, said DNA sequence is expressed in said cells and an immune response is generated against said antigen.

19. The method of claim 18 wherein said compound is bupivacaine.

20. The method of claim 18 wherein said DNA molecule is a plasmid.

21. The method of claim 18 wherein said immune response generated against said antigen is an immune response against a pathogen antigen.

22. The method of claim 21 wherein said pathogen is an intracellular pathogen.

23. The method of claim 22 wherein said pathogen is a virus selected from the group consisting of: human immunodeficiency virus, HIV; human T cell leukemia virus, HTLV; influenza virus; hepatitis a virus; hepatitis B virus; hepatitis C virus; human papilloma virus, HPV; Herpes simplex 1 virus, HSV1; Herpes simplex 2 virus, HSV2; **Cytomegalovirus, CMV;** Epstein-Barr virus, EBR; rhinovirus; and, coronavirus.

24. The method of claim 23 wherein said pathogen is HIV and said DNA molecule comprises a DNA sequence that encodes an HIV antigen.

25. The method of claim 18 wherein said immune response generated against said antigen is an immune response against a hyperproliferative disease-associated protein.

26. The method of claim 25 wherein said hyperproliferative disease-associated protein is selected from the group consisting of: protein products of oncogenes myb, myc, fyn, ras, src, neu and trk; protein products of translocation gene bcr/abl; P53; variable regions of antibodies made by B cell lymphomas; and variable regions of T cell receptors of T cell lymphomas.

27. The method of claim 18 wherein said immune response generated against said antigen is an immune response against an autoimmune disease-associated protein.

28. The method of claim 27 wherein said autoimmune disease-associated protein is selected from the group consisting of: variable regions of antibodies involved in B cell mediated autoimmune disease; and variable regions of T cell receptors involved in T cell mediated autoimmune disease.

29. The method of claim 18 wherein said bupivacaine and a DNA molecule are administered subcutaneously.

30. The method of claim 18 wherein said bupivacaine and a DNA molecule are administered intramuscularly, intraperitoneally, intravenously, intraarterially, intraoccularly, orally transdermally and/or by inhalation.

31. The method of claim 18 wherein said bupivacaine and a DNA molecule are administered intradermally.

32. The method of claim 31 wherein said immune response generated against said antigen is an immune response against a pathogen antigen.

33. The method of claim 31 wherein said immune response generated against said antigen is an immune response against a hyperproliferative disease-associated protein.

34. The method of claim 31 wherein said immune response generated

against said antigen is an immune response against an autoimmune disease-associated protein.

L23 ANSWER 10 OF 10 USPATFULL on STN
97:3820 Genetic immunization.
Weiner, David B., Merion, PA, United States
Williams, William V., Havertown, PA, United States
Wang, Bin, Havertown, PA, United States
The Wistar Institute, Philadelphia, PA, United States (U.S. corporation)The Trustees of the University of Pennsylvania, Philadelphia, PA, United States (U.S. corporation)
US 5593972 19970114
APPLICATION: US 1993-125012 19930921 (8) <--
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Methods of prophylactic and therapeutic immunization of an individual against pathogen infection, diseases associated with hyperproliferative cells and autoimmune diseases are disclosed. The methods comprise the steps of administering to cells of an individual, a nucleic acid molecule that comprises a nucleotide sequence that encodes a protein which comprises at least one epitope that is identical or substantially similar to an epitope of a pathogen antigen, a hyperproliferative cell associated protein or a protein associated with autoimmune disease respectively. In each case, nucleotide sequence is operably linked to regulatory sequences to enable expression in the cells. The nucleic acid molecule is free of viral particles and capable of being expressed in said cells. The cells may be contacted cells with a cell stimulating agent. Methods of prophylactically and therapeutically immunizing an individual against HIV are disclosed. Pharmaceutical compositions and kits for practicing methods of the present invention are disclosed.

CLM What is claimed is:

1. A method of immunizing an individual comprising: injecting into skeletal muscle tissue of said individual at a site on said individual's body, bupivacaine and a DNA molecule that comprises a DNA sequence that encodes an antigen from a pathogen, said DNA sequence operatively linked to regulatory sequences which control the expression of said DNA sequence; wherein said DNA molecule is taken up by cells in said skeletal muscle tissue, said DNA sequence is expressed in said cells and an immune response is generated against said antigen.

2. The method of claim 1 wherein said pathogen is an intracellular pathogen.

3. The method of claim 1 wherein said pathogen is a virus selected from the group consisting of: human immunodeficiency virus, HIV; human T cell leukemia virus, HTLV; influenza virus; hepatitis A virus; hepatitis B virus; hepatitis C virus; human papilloma virus, HPV; Herpes simplex 1 virus, HSV1; Herpes simplex 2 virus, HSV2; **Cytomegalovirus**, **CMV**; Epstein-Barr virus, EBR; rhinovirus; and, coronavirus.

4. The method of claim 1 wherein said pathogen is HIV and said DNA molecule comprises a DNA sequence that encodes an HIV antigen.

5. The method of claim 1 wherein at least two non-identical DNA molecules are injected into skeletal muscle tissue of said individual at different sites on said individual's body, said bupivacaine being injected into each of the different sites of an individual; said non-identical DNA molecules each comprising DNA sequences encoding one or more pathogen antigens of the same pathogen.

6. A method of immunizing an individual comprising: injecting into skeletal muscle tissue of said individual at a site on said individual's body, bupivacaine and a DNA molecule that comprises a DNA sequence that encodes a hyperproliferative disease-associated protein operatively linked to regulatory sequences; wherein said DNA molecule is taken up by

cells in said skeletal muscle tissue, said DNA sequence is expressed in said cells, and an immune response is generated against said hyperproliferative disease-associated protein.

7. The method of claim 6 wherein said DNA molecule comprises a DNA sequence encoding a target protein selected from the group consisting of: protein products of oncogenes myb, myc, fyn, ras, src, neu and trk; protein products of translocation gene bcr/abl; P53; variable regions of antibodies made by B cell lymphomas; and variable regions of T cell receptors of T cell lymphomas.

8. A method of immunizing an individual comprising: injecting into skeletal muscle tissue of said individual, bupivacaine and a DNA molecule that comprises a DNA sequence that encodes an autoimmune disease-associated protein operatively linked to regulatory sequences; wherein said DNA molecule is taken up by cells in said skeletal muscle tissue, said DNA sequence is expressed in said cells and an immune response is generated against said autoimmune disease-associated protein.

9. The method of claim 8 wherein said DNA molecule comprises a DNA sequence encoding a target protein selected from the group consisting of: variable regions of antibodies involved in B cell mediated autoimmune disease; and variable regions of T cell receptors involved in T cell mediated autoimmune disease.

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L23 ANSWER 2 OF 10 USPATFULL on STN
2001:107871 DNA vaccines against tick-borne **flaviviruses**.
Schmaljohn, Connie S., Frederick, MD, United States
The United States of America as represented by the Secretary of the Army, Washington, DC, United States (U.S. corporation)
US 6258788 B1 20010710
APPLICATION: US 1998-197218 19981120 (9) <--
PRIORITY: US 1997-65750P 19971120 (60)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Particle mediated immunization of tick-borne **flavivirus** genes confers homologous and heterologous protection against tick borne encephalitis.

CLM What is claimed is:
1. A method for inducing a protective immune response to a tick-borne **flavivirus** protein in a mammal, comprising (i) preparing a nucleic acid encoding an antigenic determinant of a tick-borne **flavivirus** prM/E protein operatively linked to a **CMV** promoter operative in cells of a mammal, which nucleic acid is suitable for stably producing the antigenic determinant in a mammal; (ii) coating the nucleic acid in (i) onto carrier particles; (iii) accelerating the coated carrier particles into epidermal cells of the mammal in vivo; and (iv) inducing a protective immune response in said mammal upon exposure to a tick-borne **flavivirus**.

2. The method according to claim 1 wherein the carrier particles are gold.

3. The method according to claim 1 wherein the tick-borne **flavivirus** prM/E protein is selected from the group consisting of Russian spring summer encephalitis prM/E proteins, and Central European encephalitis prM/E proteins.

4. The method according to claim 1 wherein the nucleic acid encodes a protein coding region comprising SEQ ID NO:1.

5. A method for inducing a protective immune response to a tick-borne

flavivirus protein in a mammal, comprising (i) preparing a nucleic acid encoding an antigenic determinant of a Russian spring summer encephalitis tick-borne **flavivirus** prM/E protein operatively linked to a promoter operative in cells of a mammal, which nucleic acid encodes a protein coding region comprising SEQ ID NO:2 and is suitable for stably producing the antigenic determinant in a mammal; (ii) coating the nucleic acid in (i) onto carrier particles; (iii) accelerating the coated carrier particles into epidermal cells of the mammal in vivo; and (iv) inducing a protective immune response in said mammal upon exposure to a tick-borne **flavivirus**.

6. The method according to claim 1 wherein the nucleic acid encodes a protein coding region comprising SEQ ID NO: 1 and SEQ ID NO:2.

7. A kit for inducing a protective immune response to a tick-borne **flavivirus** protein in a mammal, comprising packaged in association: (a) a nucleic acid encoding an antigenic determinant of a tick-borne **flavivirus** prM/E protein operatively linked to a **CMV** promoter operative in cells of a mammal, which nucleic acid is suitable for stably producing the antigenic determinant in a mammal; (b) one or both of a coating solution and/or components of a coating solution; and (c) carrier particles.

8. The kit of claim 7, wherein the tick-borne **flavivirus** prM/E protein is selected from the group consisting of Russian spring summer encephalitis prM/E proteins, and Central European encephalitis prM/E proteins.

9. The kit of claim 7, wherein the nucleic acid encodes a protein coding region comprising SEQ ID NO:1.

10. The kit of claim 7, wherein the nucleic acid encodes a protein coding region comprising SEQ ID NO:1 and SEQ ID NO:2.

11. A kit for inducing a protective immune response to a tick-borne **flavivirus** protein in a mammal, comprising packaged in association: (a) a nucleic acid encoding an antigenic determinant of a Russian spring summer encephalitis tick-borne **flavivirus** prM/E protein operatively linked to a promoter operative in cells of a mammal which nucleic acid encodes a protein coding region comprising SEQ ID NO:2 and is suitable for stably producing the antigenic determinant in a mammal; (b) one or both of a coating solution and/or components of a coating solution; and (c) carrier particles.

TI DNA vaccines against tick-borne **flaviviruses**

AI US 1998-197218 19981120 (9) <--

AB Particle mediated immunization of tick-borne **flavivirus** genes confers homologous and heterologous protection against tick borne encephalitis.

SUMM . . . over a wide area of Europe and the former Soviet Union. TBE is most frequently caused by infection with the **flaviviruses** Central European encephalitis (CEE) virus, or Russian spring summer encephalitis (RSSE) virus. These viruses are antigenically and genetically closely related. . .

SUMM . . . candidate vaccines, which express the premembrane (prM) and envelope (E) genes of RSSE or CEE viruses under control of a **cytomegalovirus** early promoter. We chose the prM and E genes for expression because of earlier reports with other **flaviviruses** which indicated that coexpressed prM and E form subviral particles that are able to elicit neutralizing and protective immune responses. . .

SUMM . . . in adverse side effects to the vaccinated individual. In addition, the invention does not require growth or use of tick-borne **flavivirus**, which may be spread by aerosol transmission and are typically fatal.

DRWD . . . are similar to those of pWRG1602 described previously (Dimmock, N. J., 1995, Med. Virol. 5: 165) and include a human **cytomegalovirus** early promoter (**CMV IE** promoter) and intron A, a bovine growth

hormone transcription terminator and polyadenylation signal (BGH pA), and a kanamycin resistance gene.

DETD In one embodiment, the present invention relates to a DNA or cDNA segment which encodes an antigen from a tick-borne **flavivirus** such as RSSE, CEE, or Langat. More specifically, prM and E genes of CEE were deduced from the CEE viral. . .

DETD . . . were modified around the translation initiation codon (bold type below) to generate sequences with a favorable context for translation initiation (**Kozak**, M., 1989, J. Cell. Biol. 108:229). The forward and reverse primers for RSSE were: 5'GCAGTAGACAGGATGGGTTGGTTG3' (SEQ ID NO:3) and 5'GCACAGCCAACTTAAGCTCCCACTCC3'. . .

DETD . . . affecting the ability of the construct to achieve the desired effect, namely induction of a protective immune response against tick-borne **flavivirus** challenge. It is further understood in the art that certain advantageous steps can be taken to increase the antigenicity of. . . by modifying the genetic sequence encoding the protein. It is contemplated that all such modifications and variations of the tick-borne **flavivirus** glycoprotein genes are equivalents within the scope of the present invention.

DETD . . . (Konishi, E. et al., 1992, Virology 188:714), or any expression vector such as viral vectors e.g. adenovirus or Venezuelan equine **encephalitis virus** and others known in the art. Preferably, a promoter sequence operable in the target cells is operably linked to the. . . 5', or upstream, of the coding sequence for the encoded protein to be expressed. A suitable promoter is the human **cytomegalovirus immediate early promoter**. A downstream transcriptional terminator, or polyadenylation sequence, such as the polyA addition sequence of the bovine growth hormone gene, may. . .

DETD . . . the method of the present invention is pWRG7077 (4326 bp)(PowderJect Vaccines, Inc., Madison, Wisc.), FIG. 1. pWRG7077 includes a human **cytomegalovirus** (hCMV) **immediate early promoter** and a bovine growth hormone polyA addition site. Between the promoter and the polyA addition site is Intron A, a sequence that naturally occurs in conjunction with the hCMV **IE** promoter that has been demonstrated to increase transcription when present on an expression plasmid. Downstream from Intron A, and between. . .

DETD . . . TBE. Mice have been used extensively as the laboratory model of choice for assessment of protective immune responses to tick-borne **flaviviruses** (Gajdosova, E. et al.,1981, Acta Virol. 25:10; Heinz, F. X. and C. Kunz, 1982, J. Biol. Stand. 10:25; Holzmann, H.. . .

DETD . . . Shope, Yale Arbovirus Research Unit, New Haven, Conn. Cell lines were obtained from the American Type Culture Collection. Central European **encephalitis virus**, strain Hypr, was isolated originally in 1953 from a TBE patient in Czechoslovakia. Russian spring summer **encephalitis virus**, strain Sofjin, was isolated originally in 1937 from a TBE patient from the Far Eastern USSR. Langat virus was isolated. . .

DETD . . . were modified around the translation initiation codon (bold type below) to generate sequences with a favorable context for translation initiation (**Kozak**, M., 1989, J. Cell. Biol. 108:229). The forward and reverse primers for RSSE were: 5'GCAGTAGACAGGATGGGTTGGTTG3' (SEQ ID NO:3) and 5'GCACAGCCAACTTAAGCTCCCACTCC3'. . .

DETD . . . RSSE or CEE prM/E cloned into pWRG7077 (FIG. 1). The two plasmids have the same control elements; i.e., a human **cytomegalovirus** early promoter and intron A, and a bovine growth hormone polyadenylation/transcription termination signal. However, pWRG7077 does not contain the SV40. . .

DETD Neutralizing antibodies correlate with protective immunity to tick-borne **flaviviruses**, as demonstrated in mice by passive transfer of neutralizing monoclonal antibodies to M and E (Heinz, F. X. et al.,. . .

DETD . . . 1992, Virology 187:290). Such subviral particles, consisting of heterodimers of prM and E, are also a by product of normal **flavivirus** morphogenesis; i.e., the so-called "slowly sedimenting hemagglutinins" (SHA) (Heinz, F. and C. Kunz, 1977, Acta Virol. 21:308; Mason, P. W.. . . and P. W. Mason, 1993, supra). So, although passively transferred

neutralizing monoclonal antibodies to E can protect animals from subsequent **flavivirus** challenge (Buckley, A. and E. A. Gould, 1985, supra; Gould and Buckley, 1986, supra; Heinz, F. X. et al., 1983,. . .
DETD . . . al., 1992, Vaccine 10:345). Consequently, although either of our DNA vaccines by itself may be sufficient for immunity to TBE-causing **flaviviruses**, it may be prudent to include both DNAs in a vaccine developed for humans.

1. A method for inducing a protective immune response to a tick-borne **flavivirus** protein in a mammal, comprising (i) preparing a nucleic acid encoding an antigenic determinant of a tick-borne **flavivirus** prM/E protein operatively linked to a **CMV** promoter operative in cells of a mammal, which nucleic acid is suitable for stably producing the antigenic determinant in a. . . of the mammal in vivo; and (iv) inducing a protective immune response in said mammal upon exposure to a tick-borne **flavivirus**.

3. The method according to claim 1 wherein the tick-borne **flavivirus** prM/E protein is selected from the group consisting of Russian spring summer encephalitis prM/E proteins, and Central European encephalitis prM/E. . .

5. A method for inducing a protective immune response to a tick-borne **flavivirus** protein in a mammal, comprising (i) preparing a nucleic acid encoding an antigenic determinant of a Russian spring summer encephalitis tick-borne **flavivirus** prM/E protein operatively linked to a promoter operative in cells of a mammal, which nucleic acid encodes a protein coding. . . of the mammal in vivo; and (iv) inducing a protective immune response in said mammal upon exposure to a tick-borne **flavivirus**.

7. A kit for inducing a protective immune response to a tick-borne **flavivirus** protein in a mammal, comprising packaged in association: (a) a nucleic acid encoding an antigenic determinant of a tick-borne **flavivirus** prM/E protein operatively linked to a **CMV** promoter operative in cells of a mammal, which nucleic acid is suitable for stably producing the antigenic determinant in a. . .

8. The kit of claim 7, wherein the tick-borne **flavivirus** prM/E protein is selected from the group consisting of Russian spring summer encephalitis prM/E proteins, and Central European encephalitis prM/E. . .

11. A kit for inducing a protective immune response to a tick-borne **flavivirus** protein in a mammal, comprising packaged in association: (a) a nucleic acid encoding an antigenic determinant of a Russian spring summer encephalitis tick-borne **flavivirus** prM/E protein operatively linked to a promoter operative in cells of a mammal which nucleic acid encodes a protein coding. . .

L23 ANSWER 5 OF 10 USPATFULL on STN
2000:18426 Chimeric hepatitis B/hepatitis C virus vaccine.
Wands, Jack R., Waban, MA, United States
Tokushige, Katsutoshi, Boston, MA, United States
Wakita, Takaji, Tokyo, Japan
The General Hospital Corporation, Charlestown, MA, United States (U.S. corporation)
US 6025341 20000215
APPLICATION: US 1997-854531 19970512 (8) <--
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Nucleic acid molecule that comprise an incomplete hepatitis C and hepatitis B viral genome including specifically disclosed DNA sequences are disclosed. Pharmaceutical compositions that contain nucleic acid molecules comprising an incomplete hepatitis C and hepatitis B viral genome including a nucleotide sequence encoding a complete hepatitis C core protein and hepatitis B S gene protein operably linked to regulatory elements functional in human cells are disclosed. Methods of immunizing individuals susceptible to or infected by hepatitis B virus and/or hepatitis C virus comprising the step of administering such

pharmaceutical compositions are disclosed.

CLM What is claimed is:

1. A recombinant DNA molecule comprising a nucleotide coding sequence that encodes a fusion protein, wherein said fusion protein consists of a hepatitis B virus S gene product linked to amino acids 1-69 of the hepatitis C virus core protein.

2. The recombinant nucleic acid molecule of claim 1 wherein the N terminal amino acid of said hepatitis C virus core protein is linked to the C terminal amino acid of said hepatitis B virus S gene product.

3. A method of inducing an immune response against hepatitis C virus in an individual uninfected by hepatitis C virus comprising the step of: administering to said individual the DNA molecule of claim 1 in an amount effective to induce an immune response against hepatitis C virus.

4. A method of treating an individual who is infected with the hepatitis C virus comprising the step of: administering to said individual the DNA molecule of claim 1 in an amount effective to induce an immune response against hepatitis C virus, wherein antibodies are produced.

5. A recombinant DNA molecule comprising a nucleotide sequence that encodes a fusion protein, wherein said fusion protein consists of a hepatitis B virus S gene product linked to amino acids 1-70 to 1-154 of the hepatitis C virus core protein.

6. The recombinant nucleic acid molecule of claim 5 wherein the N terminal amino acid of said hepatitis C virus core protein is linked to the C terminal amino acid of said hepatitis B virus S gene product.

7. A method of inducing an immune response against hepatitis C virus in an individual uninfected by hepatitis C virus comprising the step of: administering to said individual the DNA molecule of claim 5 in an amount effective to induce an immune response against hepatitis C virus.

8. A method of treating an individual who is infected with hepatitis C virus comprising the step of: administering to said individual the DNA molecule of claim 5 in an amount effective to induce an immune response against hepatitis C virus, wherein antibodies are produced.

9. A recombinant DNA molecule comprising a nucleotide sequence that encodes a fusion protein, wherein said fusion protein consists of a fragment of the hepatitis B virus pre S2 gene product linked to the hepatitis B virus S gene product linked to amino acids 1-69 of the hepatitis C virus core protein.

10. The recombinant nucleic acid molecule of claim 9 wherein the N terminal amino acid of said hepatitis B virus S gene product is linked to the C terminal amino acid of said hepatitis B virus pre S2 gene product, and the N terminal amino acid of said hepatitis C virus core protein is linked to the C terminal amino acid of said hepatitis B virus S gene product.

11. A method of inducing an immune response against hepatitis C virus in an individual uninfected by hepatitis C virus comprising the step of: administering to said individual the DNA molecule of claim 9 in an amount effective to induce an immune response against hepatitis C virus.

12. A method of treating an individual who is infected with hepatitis C virus comprising the step of: administering to said individual the DNA molecule of claim 9 in an amount effective to induce an immune response against hepatitis C virus, wherein antibodies are produced.

13. A recombinant DNA molecule comprising a nucleotide sequence that encodes a fusion protein, wherein said fusion protein consists of a fragment of the hepatitis B virus pre S2 gene product linked to the

hepatitis B virus S gene product linked to amino acids 1-70 to 1-154 of the hepatitis C virus core protein.

14. The recombinant nucleic acid molecule of claim 13 wherein the N terminal amino acid of said hepatitis B virus S gene product is linked to the C terminal amino acid of said hepatitis B virus pre S2 gene product, and the N terminal amino acid of said hepatitis C virus core protein is linked to the C terminal amino acid of said hepatitis B virus S gene product.

15. A method of inducing an immune response against hepatitis C virus in an individual uninfected by hepatitis C virus comprising the step of: administering to said individual the DNA molecule of claim 13 in an amount effective to induce an immune response against hepatitis C virus.

16. A method of treating an individual who is infected with hepatitis C virus comprising the step of: administering to said individual the DNA molecule of claim 13 in an amount effective to induce an immune response against hepatitis C virus, wherein antibodies are produced.

17. A recombinant DNA molecule comprising a nucleotide sequence that encodes a fusion protein, wherein said fusion protein consists of the hepatitis B virus pre S2 gene product linked to the hepatitis B virus S gene product linked to amino acids 1-69 of the hepatitis C virus core protein.

18. The recombinant nucleic acid molecule of claim 17 wherein the N terminal amino acid of said hepatitis B virus S gene product is linked to the C terminal amino acid of said hepatitis B virus pre S2 gene product, and the N terminal amino acid of said hepatitis C virus core protein is linked to the C terminal amino acid of said hepatitis B virus S gene product.

19. A method of immunizing an individual susceptible to or infected by hepatitis C virus for producing antibodies comprising the step of: administering to said individual the DNA molecule of claim 17 in an amount effective to induce an immune response, wherein antibodies are produced.

20. A method of treating an individual who is infected with hepatitis C virus comprising the step of: administering to said individual the DNA molecule of claim 17 in an amount effective to induce an immune response against hepatitis C virus, wherein antibodies are produced.

21. A recombinant DNA molecule comprising a nucleotide sequence that encodes a fusion protein, wherein said fusion protein consists of the hepatitis B virus pre S2 gene product linked to the hepatitis B virus S gene product linked to amino acids 1-70 to 1-154 of the hepatitis C virus core protein.

22. The recombinant nucleic acid molecule of claim 21 wherein the N terminal amino acid of said hepatitis B virus S gene product is linked to the C terminal amino acid of said hepatitis B virus pre S2 gene product, and the N terminal amino acid of said hepatitis C virus core protein is linked to the C terminal amino acid of said hepatitis B virus S gene product.

23. A method of immunizing an individual susceptible to or infected by hepatitis C virus for producing antibodies comprising the step of: administering to said individual the DNA molecule of claim 21 in an amount effective to induce an immune response, wherein antibodies are produced.

24. A method of treating an individual who is infected with hepatitis C virus comprising the step of: administering to said individual the DNA molecule of claim 21 in an amount effective to induce an immune response

against hepatitis C virus, wherein antibodies are produced.

25. The recombinant DNA molecule of any one of claims 1, 5, 9, 13, 17, or 21 comprising a **cytomegalovirus** promoter, a Rous Sarcoma Virus enhancer, and a polyadenylation sequence, wherein the nucleotide coding sequence is operatively linked to said promoter, enhancer, and polyadenylation sequence.

26. The recombinant DNA molecule of claim 25 further comprising the 5' UTR of hepatitis C virus, wherein said nucleotide coding sequence is operatively linked thereto.

27. A recombinant DNA molecule comprising a nucleotide coding sequence that encodes a fusion protein, wherein said fusion protein is selected from the group consisting of: a fusion protein that consists of the hepatitis B virus S gene product linked to a truncated hepatitis C virus core protein, a fusion protein that consists of a fragment of the the hepatitis B virus pre S2 gene product linked to the hepatitis B virus S gene product linked to a truncated hepatitis C virus core protein, and a fusion protein that consists of the hepatitis B virus pre S2 gene product linked to the hepatitis B virus S gene product linked to a truncated hepatitis C virus core protein.

28. The recombinant DNA molecule of claim 27 wherein said fusion protein consists of the hepatitis B virus S gene product linked to a truncated hepatitis C virus core protein.

29. The recombinant DNA molecule of claim 27 wherein said fusion protein consists of a fragment of the hepatitis B virus pre S2 gene product linked to the hepatitis B virus S gene product linked to a truncated hepatitis C virus core protein.

30. The recombinant DNA molecule of claim 27 wherein said fusion protein consists of the hepatitis B virus pre S2 gene product linked to the hepatitis B virus S gene product linked to a truncated hepatitis C virus core protein.

31. A pharmaceutical composition comprising: a) a recombinant DNA molecule of claim 1; wherein said nucleotide sequence is operably linked to regulatory elements functional in human cells; and b) a pharmaceutically acceptable carrier or diluent.

32. The pharmaceutical composition of claim 31 wherein said regulatory elements functional in human cells comprise a **cytomegalovirus** promoter, a Rous Sarcoma Virus enhancer, a polyadenylation sequence.

33. The pharmaceutical composition of claim 32 further comprising the 5' UTR of hepatitis C virus.

34. A method of immunizing an individual susceptible to or infected by hepatitis C virus for producing antibodies comprising the step of: administering to said individual the pharmaceutical composition of claim 31 in an amount effective to induce an immune response, wherein antibodies are produced.

35. The method of claim 34 wherein bupivacaine is administered to said individual at the site of administration of the pharmaceutical composition.

36. A pharmaceutical composition comprising: a) the recombinant DNA molecule of claim 5, wherein said nucleotide sequence is operably linked to regulatory elements functional in human cells; and b) a pharmaceutically acceptable carrier or diluent.

37. The pharmaceutical composition of claim 36 wherein said regulatory elements functional in human cells comprise a **cytomegalovirus**

promoter, a Rous Sarcoma Virus enhancer, a polyadenylation sequence.

38. The pharmaceutical composition of claim 37 further comprising the 5' UTR of hepatitis C virus.

39. A method of immunizing an individual susceptible to or infected by hepatitis C virus for producing antibodies comprising the step of: administering to said individual the pharmaceutical composition of claim 36 in an amount effective to induce an immune response, wherein antibodies are produced.

40. The method of claim 39 wherein bupivacaine is administered to said individual at the site of administration of the pharmaceutical composition.

41. A pharmaceutical composition comprising: a) the recombinant DNA molecule of claim 9, wherein said nucleotide sequence is operably linked to regulatory elements functional in human cells; and b) a pharmaceutically acceptable carrier or diluent.

42. The pharmaceutical composition of claim 41 wherein said regulatory elements functional in human cells comprise a **cytomegalovirus** promoter, a Rous Sarcoma Virus enhancer, a polyadenylation sequence.

43. The pharmaceutical composition of claim 42 further comprising the 5' UTR of hepatitis C virus.

44. A method of immunizing an individual susceptible to or infected by hepatitis C virus for producing antibodies comprising the step of: administering to said individual the pharmaceutical composition of claim 41 in an amount effective to induce an immune response, wherein antibodies are produced.

45. The method of claim 44 wherein bupivacaine is administered to said individual at the site of administration of the pharmaceutical composition.

46. A pharmaceutical composition comprising: a) the recombinant DNA molecule of claim 13, wherein said nucleotide sequence is operably linked to regulatory elements functional in human cells; and b) a pharmaceutically acceptable carrier or diluent.

47. The pharmaceutical composition of claim 46 wherein said regulatory elements functional in human cells comprise a **cytomegalovirus** promoter, a Rous Sarcoma Virus enhancer, a polyadenylation sequence.

48. The pharmaceutical composition of claim 47 further comprising the 5' UTR of hepatitis C virus.

49. A method of immunizing an individual susceptible to or infected by hepatitis C virus for producing antibodies comprising the step of: administering to said individual the pharmaceutical composition of claim 46 in an amount effective to induce an immune response, wherein antibodies are produced.

50. The method of claim 49 wherein bupivacaine is administered to said individual at the site of administration of the pharmaceutical composition.

51. A pharmaceutical composition comprising: a) the recombinant DNA molecule of claim 17, wherein said nucleotide sequence is operably linked to regulatory elements functional in human cells; and b) a pharmaceutically acceptable carrier or diluent.

52. The pharmaceutical composition of claim 51 wherein said regulatory elements functional in human cells comprise a **cytomegalovirus**

promoter, a Rous Sarcoma Virus enhancer, a polyadenylation sequence.

53. The pharmaceutical composition of claim 52 further comprising the 5' UTR of hepatitis C virus.

54. A method of immunizing an individual susceptible to or infected by hepatitis C virus for producing antibodies comprising the step of: administering to said individual the pharmaceutical composition of claim 51 in an amount effective to induce an immune response, wherein antibodies are produced.

55. The method of claim 54 wherein bupivacaine is administered to said individual at the site of administration of the pharmaceutical composition.

56. A pharmaceutical composition comprising: a) the recombinant DNA molecule of claim 21, wherein said nucleotide sequence is operably linked to regulatory elements functional in human cells; and b) a pharmaceutically acceptable carrier or diluent.

57. The pharmaceutical composition of claim 56 wherein said regulatory elements functional in human cells comprise a **cytomegalovirus** promoter, a Rous Sarcoma Virus enhancer, a polyadenylation sequence.

58. The pharmaceutical composition of claim 57 further comprising the 5' UTR of hepatitis C virus.

59. A method of immunizing an individual susceptible to or infected by hepatitis C virus for producing antibodies comprising the step of: administering to said individual the pharmaceutical composition of claim 56 in an amount effective to induce an immune response, wherein antibodies are produced.

60. The method of claim 59 wherein bupivacaine is administered to said individual at the site of administration of the pharmaceutical composition.

AI US 1997-854531 19970512 (8) <--

SUMM . . . positive stranded RNA virus, approximately 9,500 nucleotides in length, which has recently been classified as a separate genus within the **Flavivirus** family (Heinz, F. X., Arch. Virol. (Suppl.), 1992, 4, 163-171). Different isolates show considerable nucleotide sequence diversity leading to the. . .

DETD . . . of directing expression in the cells of the vaccinated individual. In some embodiments, the gene construct further comprises an enhancer, **Kozak** sequence (GCCGCCATG SEQ ID NO:13), and at least a fragment of the HCV 5' UTR.

DETD . . . The regulatory elements include a promoter and a polyadenylation signal. In addition, other elements, such as an enhancer and a **Kozak** sequence, may also be included in the gene construct.

DETD . . . Tumor Virus (MMTV) promoter, Human Immunodeficiency Virus (HIV) such as the HIV Long Terminal Repeat (LTR) promoter, Moloney virus, ALV, **Cytomegalovirus** (**CMV**) such as the **CMV immediate early promoter**, Epstein Barr Virus (EBV), Rous Sarcoma Virus (RSV) as well as promoters from human genes such as human Actin, human. . .

DETD . . . but not limited to: human Actin, human Myosin, human Hemoglobin, human muscle creatine and viral enhancers such as those from **CMV**, RSV and EBV.

DETD In expression vectors of the invention, nucleotide coding sequence encoding the fusion protein is under the regulatory control of the **CMV immediate early promoter** and the SV40 minor polyadenylation signal. Constructs may optionally contain the SV40 origin of replication.

DETD . . . (pre S2-S) PCR product by Xho-I followed by Klenow treatment. In the upstream sequence of the pre-S2-S-HCV fusion constructs, a **Kozak** sequence (GCCGCCATG SEQ ID NO:13) was included in the Kz Hind

pb2 primer and this was added to the pres2 5 nov. . .

DETD . . . proteins described above each contain the nucleotide coding region for the fusion protein placed under the transcriptional control of the **CMV** promoter and the RSV enhancer element.

DETD . . . and operably linked to the promoter and polyadenylation signal. Transcription of the cloned inserts is under the control of the **CMV** promoter and the RSV enhancer elements. A polyadenylation signal is provided by the presence of an SV40 poly A signal. . .

25. The recombinant DNA molecule of any one of claims 1, 5, 9, 13, 17, or 21 comprising a **cytomegalovirus** promoter, a Rous Sarcoma Virus enhancer, and a polyadenylation sequence, wherein the nucleotide coding sequence is operatively linked to said. . .

32. The pharmaceutical composition of claim 31 wherein said regulatory elements functional in human cells comprise a **cytomegalovirus** promoter, a Rous Sarcoma Virus enhancer, a polyadenylation sequence.

37. The pharmaceutical composition of claim 36 wherein said regulatory elements functional in human cells comprise a **cytomegalovirus** promoter, a Rous Sarcoma Virus enhancer, a polyadenylation sequence.

42. The pharmaceutical composition of claim 41 wherein said regulatory elements functional in human cells comprise a **cytomegalovirus** promoter, a Rous Sarcoma Virus enhancer, a polyadenylation sequence.

47. The pharmaceutical composition of claim 46 wherein said regulatory elements functional in human cells comprise a **cytomegalovirus** promoter, a Rous Sarcoma Virus enhancer, a polyadenylation sequence.

52. The pharmaceutical composition of claim 51 wherein said regulatory elements functional in human cells comprise a **cytomegalovirus** promoter, a Rous Sarcoma Virus enhancer, a polyadenylation sequence.

57. The pharmaceutical composition of claim 56 wherein said regulatory elements functional in human cells comprise a **cytomegalovirus** promoter, a Rous Sarcoma Virus enhancer, a polyadenylation sequence.

=> d hs
'HS' IS NOT A VALID FORMAT FOR FILE 'USPATFULL'

The following are valid formats:

The default display format is STD.

ABS -------- AB
ALL -------- AN, TI, IN, INA, PA, PAA, PAT, PI, AI, PTERM, DCD, RLI, PRAI, DT, FS, REP, REN, EXNAM, LREP, CLMN, ECL, DRWN, AB, GOVI, PARN, SUMM, DRWD, DETD, CLM, INCL, INCLM, INCLS, NCL, NCLM, NCLS, IC, ICM, ICS, EXF, ARTU
ALLG ------- ALL plus PAGE.DRAW
BIB -------- AN, TI, IN, INA, PA, PAA, PAT, PI, AI, PTERM, DCD, RLI, PRAI, DT, FS, EXNAM, LREP, CLMN, ECL, DRWN, LN.CNT
BIB.EX ----- BIB for original and latest publication
BIBG ------- BIB plus PAGE.DRAW
BROWSE ----- See "HELP BROWSE" or "HELP DISPLAY BROWSE". BROWSE must entered on the same line as DISPLAY, e.g., D BROWSE.
CAS -------- OS, CC, SX, ST, IT
CBIB ------- AN, TI, IN, INA, PA, PAA, PAT, PI, AI, PRAI, DT, FS
DALL ------- ALL, delimited for post-processing
FP --------- PI, TI, IN, INA, PA, PAA, PAT, PTERM, DCD, AI, RLI, PRAI, IC, ICM, ICS, INCL, INCLM, INCLS, NCL, NCLM, NCLS, EXF, REP, REN, ARTU, EXNAM, LREP, CLMN, DRWN, AB
FP.EX ------ FP for original and latest publication

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FFABB          PI, TI, IN, INA, PA, PAA, PAT, PTERM, DCD, AI,
               RLI, PRAI, IC, ICM, ICS, INCL, INCLM, INCLS, NCL, NCLM,
               NCLS, EXF, REP, REN,ARTU, EXNAM, LREP, CLMN, DRWN, AB,
               PARN, SUMM, DRWD, DETD, CLM
FPBIB ------ PI, TI, IN, INA, PA, PAA, PAT, PTERM, DCD, AI,
               RLI, PRAI, REP, REN, EXNAM, LREP, CLM, CLMN, DRWN
FHITSTR ---- HIT RN, its text modification, its CA index name, and
               its structure diagram
FPG -------- FP plus PAGE.DRAW
GI --------- PN and page image numbers
HIT -------- All fields containing hit terms
HITRN ------ HIT RN and its text modification
HITSTR ----- HIT RN, its text modification, its CA index name, and
               its structure diagram
IABS ------- ABS, indented with text labels
IALL ------- ALL, indented with text labels
IALLG ------ IALL plus PAGE.DRAW
IBIB ------- BIB, indented with text labels
IBIB.EX ---- IBIB for original and latest publication
IBIBG ------ IBIB plus PAGE.DRAW
IMAX ------- MAX, indented with text labels
IMAX.EX ---- IMAX for original and latest publication
IND -------- INCL, INCLM, INCLS, NCL, NCLM, NCLS, IC, ICM, ICS,
               EXF, ARTU, OS, CC, SX, ST, IT
ISTD ------- STD, indented with text labels
KWIC ------- All hit terms plus 20 words on either side
MAX -------- AN, TI, IN, INA, PA, PAA, PAT, PI, AI, PTERM, DCD,
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               INCLM, INCLS, NCL, NCLM, NCLS, IC, ICM, ICS,
               EXF, ARTU OS, CC, SX, ST, IT
MAX.EX ----- MAX for original and latest publication
OCC -------- List of display fields containing hit terms
SBIB ------- AN, TI, IN, INA, PA, PAA, PAT, PI, AI, RLI, PRAI,
               DT, FS, LN.CNT
SCAN ------- AN, TI, NCL, NCLM, NCLS, IC, ICM, ICS (random display
               without answer number.  SCAN must be entered on the
               same line as DISPLAY, e.g., D SCAN)
STD -------- AN, TI, IN, INA, PA, PAA, PAT, PI, AI, RLI, PRAI,
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STD.EX ----- STD for original and latest publication
TRIAL ------ AN, TI, INCL, INCLM, INCLS, NCL, NCLM, NCLS, IC,
               ICM, ICS

ENTER DISPLAY FORMAT (STD):d his
'D' IS NOT A VALID FORMAT FOR FILE 'USPATFULL'
'HIS' IS NOT A VALID FORMAT FOR FILE 'USPATFULL'

The following are valid formats:

The default display format is STD.

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               INCLM, INCLS, NCL, NCLM, NCLS, IC, ICM, ICS,
               EXF, ARTU
ALLG ------- ALL plus PAGE.DRAW
BIB -------- AN, TI, IN, INA, PA, PAA, PAT, PI, AI, PTERM, DCD, RLI,
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BIB.EX ----- BIB for original and latest publication
BIBG ------- BIB plus PAGE.DRAW
BROWSE ----- See "HELP BROWSE" or "HELP DISPLAY BROWSE".  BROWSE must
               entered on the same line as DISPLAY, e.g., D BROWSE.
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CAB            OS, CC, SX, ST, IT
CBIB ------- AN, TI, IN, INA, PA, PAA, PAT, PI, AI, PRAI, DT, FS
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             CLMN, DRWN, AB
FP.EX ------ FP for original and latest publication
FPALL ------ PI, TI, IN, INA, PA, PAA, PAT, PETRM, DCD, AI,
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             PARN, SUMM, DRWD, DETD, CLM
FPBIB ------ PI, TI, IN, INA, PA, PAA, PAT, PTERM, DCD, AI,
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FHITSTR ---- HIT RN, its text modification, its CA index name, and
             its structure diagram
FPG -------- FP plus PAGE.DRAW
GI --------- PN and page image numbers
HIT -------- All fields containing hit terms
HITRN ------ HIT RN and its text modification
HITSTR ----- HIT RN, its text modification, its CA index name, and
             its structure diagram
IABS ------- ABS, indented with text labels
IALL ------- ALL, indented with text labels
IALLG ------ IALL plus PAGE.DRAW
IBIB ------- BIB, indented with text labels
IBIB.EX ---- IBIB for original and latest publication
IBIBG ------ IBIB plus PAGE.DRAW
IMAX ------- MAX, indented with text labels
IMAX.EX ---- IMAX for original and latest publication
IND -------- INCL, INCLM, INCLS, NCL, NCLM, NCLS, IC, ICM, ICS,
             EXF, ARTU, OS, CC, SX, ST, IT
ISTD ------- STD, indented with text labels
KWIC ------- All hit terms plus 20 words on either side
MAX -------- AN, TI, IN, INA, PA, PAA, PAT, PI, AI, PTERM, DCD,
             RLI, PRAI, DT, FS, REP, REN, EXNAM, LREP, CLMN, ECL,
             DRWN, AB, GOVI, PARN, SUMM, DRWD, DETD, CLM, INCL,
             INCLM, INCLS, NCL, NCLM, NCLS, IC, ICM, ICS,
             EXF, ARTU OS, CC, SX, ST, IT
MAX.EX ----- MAX for original and latest publication
OCC -------- List of display fields containing hit terms
SBIB ------- AN, TI, IN, INA, PA, PAA, PAT, PI, AI, RLI, PRAI,
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SCAN ------- AN, TI, NCL, NCLM, NCLS, IC, ICM, ICS (random display
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             same line as DISPLAY, e.g., D SCAN)
STD -------- AN, TI, IN, INA, PA, PAA, PAT, PI, AI, RLI, PRAI,
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STD.EX ----- STD for original and latest publication
TRIAL ------ AN, TI, INCL, INCLM, INCLS, NCL, NCLM, NCLS, IC,
             ICM, ICS

ENTER DISPLAY FORMAT (STD):ti

L23  ANSWER 1 OF 10  USPATFULL on STN
TI     COMPOSITIONS AND METHODS FOR TREATING INFECTIONS USING CATIONIC PEPTIDES
       ALONE OR IN COMBINATION WITH ANTIBIOTICS


=> d his

     (FILE 'HOME' ENTERED AT 15:58:13 ON 30 APR 2004)

     FILE 'USPATFULL' ENTERED AT 15:59:07 ON 30 APR 2004
               E CHANG GWONG JEN/IN
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L1              1 S E4

     FILE 'WPIDS' ENTERED AT 16:00:09 ON 30 APR 2004
                E CHANG G J/IN
L2            106 S E3
L3              2 S L2 AND FLAVIVIR?

     FILE 'MEDLINE' ENTERED AT 16:01:13 ON 30 APR 2004
                E CHANG G J/AU
L4             49 S E3
L5             29 S L4 AND (FLAVIVIR? OR YELLOW OR DENGUE OR ENCEPHALITIS)
L6              9 S L5 AND (PRM OR E OR PRM/M OR PRM/E)

     FILE 'USPATFULL' ENTERED AT 16:06:37 ON 30 APR 2004
L7           3470 S (FLAVIVIR? OR YELLOW FEVER VIR? OR DENGUE OR ENCEPHALITIS VIR
L8             79 S L7 AND (PRM OR PREMEMBRANE)
L9             79 S L8 AND (E OR ENVELOPE)
L10            79 S L9 AND (M OR MEMBRANE)
L11            43 S L10 AND (SIGNAL SEQUENCE)
L12             5 S L11 AND KOZAK
L13            38 S L11 NOT L12
L14            15 S L13 AND AY<1999
L15           805 S L7 AND (KOZAK OR RIBOSOME BINDING SITE)
L16             2 S L15 AND KOZAK/CLM
L17             0 S L15 AND (KOZAK RIBOSOME BINDING SITE? OR KOZAK CONCENSUS SEQU
L18           716 S L15 AND (CMV OR CYTOMEGALOVIRUS)
L19           191 S L18 AND (IE OR IMMEDIATE-EARLY PROMOTER?)
L20            30 SS L19 AND (CMV/CLM OR CYTOMEGALOVIRUS/CLM)
L21             2 S L20 AND (IE/CLM OR IMMEDIATE-EARLY/CLM)
L22            28 S L20 NOT L21
L23            10 S L20 AND AY<1999

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         22836 POLYADENYLATION
        206203 POLY
       3774388 A
         17720 POLY-A
                 (POLY(W)A)
L24        743 L15 AND (POLYADENYLATION OR POLY-A)

=> s l24 and (polyadenylation (5w) terminat?)
         22836 POLYADENYLATION
        668476 TERMINAT?
          4344 POLYADENYLATION (5W) TERMINAT?
L25         423 L24 AND (POLYADENYLATION (5W) TERMINAT?)

=> s l25 and l23
L26             2 L25 AND L23

=> d l26,cbib,1-2

L26  ANSWER 1 OF 2  USPATFULL on STN
2001:107871 DNA vaccines against tick-borne flaviviruses.
    Schmaljohn, Connie S., Frederick, MD, United States
    The United States of America as represented by the Secretary of the Army,
    Washington, DC, United States (U.S. corporation)
    US 6258788 B1 20010710
    APPLICATION: US 1998-197218  19981120 (9)                    <--
    PRIORITY: US 1997-65750P  19971120 (60)
    DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L26  ANSWER 2 OF 2  USPATFULL on STN
2001:44013 Lentiviral vectors.
    Chang, Lung-Ji, 3102 NW. 57th Terr., Gainesville, FL, United States
    32606-6939
```

US 6207455 B1 20010327
APPLICATION: US 1997-935312 19970922 (8) <--
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

=> d l26,cbib,ab,kwic,1-2

L26 ANSWER 1 OF 2 USPATFULL on STN
2001:107871 DNA vaccines against tick-borne **flaviviruses**.
Schmaljohn, Connie S., Frederick, MD, United States
The United States of America as represented by the Secretary of the Army, Washington, DC, United States (U.S. corporation)
US 6258788 B1 20010710
APPLICATION: US 1998-197218 19981120 (9) <--
PRIORITY: US 1997-65750P 19971120 (60)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Particle mediated immunization of tick-borne **flavivirus** genes confers homologous and heterologous protection against tick borne encephalitis.
TI DNA vaccines against tick-borne **flaviviruses**
AI US 1998-197218 19981120 (9) <--
AB Particle mediated immunization of tick-borne **flavivirus** genes confers homologous and heterologous protection against tick borne encephalitis.
SUMM . . . over a wide area of Europe and the former Soviet Union. TBE is most frequently caused by infection with the **flaviviruses** Central European encephalitis (CEE) virus, or Russian spring summer encephalitis (RSSE) virus. These viruses are antigenically and genetically closely related. . .
SUMM . . . candidate vaccines, which express the premembrane (prM) and envelope (E) genes of RSSE or CEE viruses under control of a **cytomegalovirus** early promoter. We chose the prM and E genes for expression because of earlier reports with other **flaviviruses** which indicated that coexpressed prM and E form subviral particles that are able to elicit neutralizing and protective immune responses. . .
SUMM . . . in adverse side effects to the vaccinated individual. In addition, the invention does not require growth or use of tick-borne **flavivirus**, which may be spread by aerosol transmission and are typically fatal.
DRWD . . . are similar to those of pWRG1602 described previously (Dimmock, N. J., 1995, Med. Virol. 5: 165) and include a human **cytomegalovirus** early promoter (**CMV IE** promoter) and intron A, a bovine growth hormone transcription terminator and **polyadenylation** signal (BGH pA), and a kanamycin resistance gene.
DETD In one embodiment, the present invention relates to a DNA or cDNA segment which encodes an antigen from a tick-borne **flavivirus** such as RSSE, CEE, or Langat. More specifically, prM and E genes of CEE were deduced from the CEE viral. . .
DETD . . . were modified around the translation initiation codon (bold type below) to generate sequences with a favorable context for translation initiation (**Kozak**, M., 1989, J. Cell. Biol. 108:229). The forward and reverse primers for RSSE were: 5'GCAGTAGACAGGATGGGTTGGTTG3' (SEQ ID NO:3) and 5'GCACAGCCAACTTAAGCTCCCACTCC3'. . .
DETD . . . affecting the ability of the construct to achieve the desired effect, namely induction of a protective immune response against tick-borne **flavivirus** challenge. It is further understood in the art that certain advantageous steps can be taken to increase the antigenicity of. . . by modifying the genetic sequence encoding the protein. It is contemplated that all such modifications and variations of the tick-borne **flavivirus** glycoprotein genes are equivalents within the scope of the present invention.
DETD . . . (Konishi, E. et al., 1992, Virology 188:714), or any expression vector such as viral vectors e.g. adenovirus or Venezuelan equine **encephalitis virus** and others known in the art. Preferably, a promoter sequence operable in the target cells is operably linked to the. . . 5', or upstream, of the coding sequence for the encoded

protein to be expressed. A suitable promoter is the human **cytomegalovirus immediate early promoter**. A downstream transcriptional terminator, or **polyadenylation** sequence, such as the polyA addition sequence of the bovine growth hormone gene, may also be added 3' to the. . .

DETD . . . the method of the present invention is pWRG7077 (4326 bp)(PowderJect Vaccines, Inc., Madison, Wisc.), FIG. 1. pWRG7077 includes a human **cytomegalovirus** (hCMV) **immediate early promoter** and a bovine growth hormone polyA addition site. Between the promoter and the polyA addition site is Intron A, a sequence that naturally occurs in conjunction with the hCMV **IE** promoter that has been demonstrated to increase transcription when present on an expression plasmid. Downstream from Intron A, and between. . .

DETD . . . TBE. Mice have been used extensively as the laboratory model of choice for assessment of protective immune responses to tick-borne **flaviviruses** (Gajdosova, E. et al.,1981, Acta Virol. 25:10; Heinz, F. X. and C. Kunz, 1982, J. Biol. Stand. 10:25; Holzmann, H.. . .

DETD . . . Shope, Yale Arbovirus Research Unit, New Haven, Conn. Cell lines were obtained from the American Type Culture Collection. Central European **encephalitis virus**, strain Hypr, was isolated originally in 1953 from a TBE patient in Czechoslovakia. Russian spring summer **encephalitis virus**, strain Sofjin, was isolated originally in 1937 from a TBE patient from the Far Eastern USSR. Langat virus was isolated. . .

DETD . . . were modified around the translation initiation codon (bold type below) to generate sequences with a favorable context for translation initiation (**Kozak**, M., 1989, J. Cell. Biol. 108:229). The forward and reverse primers for RSSE were: 5'GCAGTAGACAGGATGGGTTGGTTG3' (SEQ ID NO:3) and 5'GCACAGCCAACTTAAGCTCCCACTCC3'. . .

DETD . . . RSSE or CEE prM/E cloned into pWRG7077 (FIG. 1). The two plasmids have the same control elements; i.e., a human **cytomegalovirus** early promoter and intron A, and a bovine growth hormone **polyadenylation**/transcription **termination** signal. However, pWRG7077 does not contain the SV40 virus origin of replication and it has a kanamycin resistance gene rather. . .

DETD Neutralizing antibodies correlate with protective immunity to tick-borne **flaviviruses**, as demonstrated in mice by passive transfer of neutralizing monoclonal antibodies to M and E (Heinz, F. X. et al.,. . .

DETD . . . 1992, Virology 187:290). Such subviral particles, consisting of heterodimers of prM and E, are also a by product of normal **flavivirus** morphogenesis; i.e., the so-called "slowly sedimenting hemagglutinins" (SHA) (Heinz, F. and C. Kunz, 1977, Acta Virol. 21:308; Mason, P. W.. . . and P. W. Mason, 1993, supra). So, although passively transferred neutralizing monoclonal antibodies to E can protect animals from subsequent **flavivirus** challenge (Buckley, A. and E. A. Gould, 1985, supra; Gould and Buckley, 1986, supra; Heinz, F. X. et al., 1983,. . .

DETD . . . al., 1992, Vaccine 10:345). Consequently, although either of our DNA vaccines by itself may be sufficient for immunity to TBE-causing **flaviviruses**, it may be prudent to include both DNAs in a vaccine developed for humans.

CLM What is claimed is:

1. A method for inducing a protective immune response to a tick-borne **flavivirus** protein in a mammal, comprising (i) preparing a nucleic acid encoding an antigenic determinant of a tick-borne **flavivirus** prM/E protein operatively linked to a **CMV** promoter operative in cells of a mammal, which nucleic acid is suitable for stably producing the antigenic determinant in a. . . of the mammal in vivo; and (iv) inducing a protective immune response in said mammal upon exposure to a tick-borne **flavivirus**.

3. The method according to claim 1 wherein the tick-borne **flavivirus** prM/E protein is selected from the group consisting of Russian spring summer encephalitis prM/E proteins, and Central European encephalitis prM/E. . .

5. A method for inducing a protective immune response to a tick-borne

flavivirus protein in a mammal, comprising (i) preparing a nucleic acid encoding an antigenic determinant of a Russian spring summer encephalitis tick-borne **flavivirus** prM/E protein operatively linked to a promoter operative in cells of a mammal, which nucleic acid encodes a protein coding. . . of the mammal in vivo; and (iv) inducing a protective immune response in said mammal upon exposure to a tick-borne **flavivirus**.

7. A kit for inducing a protective immune response to a tick-borne **flavivirus** protein in a mammal, comprising packaged in association: (a) a nucleic acid encoding an antigenic determinant of a tick-borne **flavivirus** prM/E protein operatively linked to a **CMV** promoter operative in cells of a mammal, which nucleic acid is suitable for stably producing the antigenic determinant in a. . .
8. The kit of claim 7, wherein the tick-borne **flavivirus** prM/E protein is selected from the group consisting of Russian spring summer encephalitis prM/E proteins, and Central European encephalitis prM/E. . .
11. A kit for inducing a protective immune response to a tick-borne **flavivirus** protein in a mammal, comprising packaged in association: (a) a nucleic acid encoding an antigenic determinant of a Russian spring summer encephalitis tick-borne **flavivirus** prM/E protein operatively linked to a promoter operative in cells of a mammal which nucleic acid encodes a protein coding. . .

L26 ANSWER 2 OF 2 USPATFULL on STN
2001:44013 Lentiviral vectors.
Chang, Lung-Ji, 3102 NW. 57th Terr., Gainesville, FL, United States 32606-6939
US 6207455 B1 20010327
APPLICATION: US 1997-935312 19970922 (8) <--
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention contemplates novel lentiviral vectors which exhibit strong promoter activity in human and other cells. Vectors are provided which are packaged efficiently in packaging cells and cell lines to generate high titer recombinant virus stocks. The present invention further relates to HIV vaccines and compositions for gene therapy. In particular, the present invention provides attenuated replication-competent HIV vaccines and replication-defective HIV vectors.
AI US 1997-935312 19970922 (8) <--
SUMM . . . addition, the promoter present in the M-MuLV LTR is quite weak compared with other viral promoters such as the human **cytomegalovirus** immediate early (**CMV-IE**) enhancer/promoter. In order to increase expression of the genes carried on the retroviral vector, internal promoters possessing stronger activities than. . .
SUMM . . . consisting of human immunodeficiency virus type 1, human immunodeficiency virus type 2, feline immunodeficiency virus, simian immunodeficiency virus, visna-maedi, caprine arthritis-**encephalitis virus**, equine infectious anemia virus, and bovine immune deficiency virus.
SUMM . . . consisting of human immunodeficiency virus type 1, human immunodeficiency virus type 2, feline immunodeficiency virus, simian immunodeficiency virus, visna-maedi, caprine arthritis-**encephalitis virus**, equine infectious anemia virus, and bovine immune deficiency virus. Thus, the recombinant lentivirus may be recombinant HIV-1, HIV-2, SIV, or. . . other embodiments, the lentiviral vector further comprises plasmid DNA selected from the group consisting of pHP-1, pHP-dl.2 and pHP-dl.28, pHP-VSVG, pHP-**CMV**, pHP-CMVdel.TAR/SD, pHP-**CMV**-EF1α intron, and pHP-EF.
SUMM . . . cell and/or cell line contains a transducing vector is selected from the group consisting of pTVψ, pTVψ100, pTVψ140, pTV.ψ.nlacZ, and pTVψ**CMV**-nlacZ-hyg-dl.SmaI, pTVΔ, pTVΔ-X, pTVΔ**CMV**-X, pTVΔCMVnlacZ, pTVΔSVneo,

pTVΔSVhyg, pTVΔ**CMV**-GFP, pTVΔ**CMV**-nlacZ, and pTVΔ**CMV**-nlacZ-hyg. In yet other embodiments, the packaging cell produces replication-defective lentivirus particles. In another embodiment, the packaging cell and/or cell line. . .

SUMM . . . consisting of human immunodeficiency virus type 1, human immunodeficiency virus type 2, feline immunodeficiency virus, simian immunodeficiency virus, visna-maedi, caprine arthritis-**encephalitis virus**, equine infectious anemia virus, and bovine immune deficiency virus. Thus, the attenuated virus may be an attenuated HIV-1, attenuated HIV-2,. . .

DRWD FIG. 1D provides simplified schematic illustrations of three heterologous enhancer/promoter inserts (human **CMV IE**(a), human **CMV IE**(b), and Mo-MLV).

DETD . . . molecules having a stretch of adenine nucleotides at the 3' end. this polyadenine stretch is also referred to as a "**poly-A** tail". Eukaryotic mRNA molecules contain **poly-A** tails and are referred to as poly^{A}+ RNA.

DETD . . . the provirus are structures called "long terminal repeats" or "LTRs." The LTR contains numerous regulatory signals including transcriptional control elements, **polyadenylation** signals and sequences needed for replication and integration of the viral genome. The viral LTR is divided into three regions. . .

DETD The U3 region contains the enhancer and promoter elements. The U5 region contains the **polyadenylation** signals. The R (repeat) region separates the U3 and U5 regions and transcribed sequences of the R region appear at. . .

DETD . . . agent of the human acquired immunodeficiency syndrome (AIDS); visna-maedi, which causes encephalitis (visna) or pneumonia (maedi) in sheep, the caprine arthritis-**encephalitis virus**, which causes immune deficiency, arthritis, and encephalopathy in goats; equine infectious anemia virus, which causes autoimmune hemolytic anemia, and encephalopathy. . .

DETD . . . base pairs in length. LTRs often provide functions fundamental to the expression of most eukaryotic genes (e.g., promotion, initiation and **polyadenylation** of transcripts).

DETD . . . particular host organism. Nucleic acid sequences necessary for expression in prokaryotes usually include a promoter, an operator (optional), and a **ribosome binding site**, often along with other sequences. Eukaryotic cells are known to utilize promoters, enhancers, and termination and **polyadenylation** signals. In some embodiments, "expression vectors" are used in order to permit pseudotyping of the viral envelope proteins.

DETD In the present invention, various transducing vectors may be used, including pTVψ, pTVψ100, pTVψ140, pTV.ψ.nlacZ, and pTVψ**CMV**-nlacZ-hyg-dl.SmaI, pTVΔ, pTVΔ-X, pTVΔ**CMV**-X, pTVΔCMVnlacZ, pTVΔSVneo, pTVΔSVhyg, pTVΔ**CMV**-GFP, pTVΔ**CMV**-nlacZ, and pTVΔ**CMV**-nlacZ-hyg. However, it is not intended that the present invention be limited to these specific transducing vectors. For example, the "pTVΔ-X,". . .

DETD . . . elements can exert their effect even when located 3' of the promoter element and the coding region. Transcription termination and **polyadenylation** signals are located 3' or downstream of the coding region.

DETD . . . region may be present in either a cDNA or genomic DNA form. Suitable control elements such as enhancers/promoters, splice junctions, **polyadenylation** signals, etc. may be placed in close proximity to the coding region of the gene if needed to permit proper. . . the coding region utilized in the expression vectors of the present invention may contain endogenous enhancers/promoters, splice junctions, intervening sequences, **polyadenylation** signals, etc. or a combination of both endogenous and exogenous control elements.

DETD . . . efficient initiation and termination. For example, a segment of DNA comprising an enhancer/promoter, a coding region and a termination and **polyadenylation** sequence comprises a transcription unit.

DETD . . . regulatory element which facilitates the initiation of transcription of an operably linked coding region. Other regulatory elements are splicing signals, **polyadenylation** signals, **termination** signals, etc. (defined infra).

DETD . . . of the Rous sarcoma virus (C. M. Gorman et al., Proc. Natl. Acad. Sci. USA 79:6777 [1982]) and the human **cytomegalovirus** (M. Boshart et al., Cell 41:521 [1985]).

DETD . . . infected cell or in a cell expressing the viral factor). The level of activity in the presence of the factor (**ie**., activity "induced" by the factor) will be higher than the basal rate.

DETD Efficient expression of recombinant DNA sequences in eukaryotic cells requires expression of signals directing the efficient termination and **polyadenylation** of the resulting transcript. Transcription **termination** signals are generally found downstream of the **polyadenylation** signal and are a few hundred nucleotides in length. The term "**poly A** site" or "**poly A** sequence" as used herein denotes a DNA sequence which directs both the termination and **polyadenylation** of the nascent RNA transcript. Efficient **polyadenylation** of the recombinant transcript is desirable as transcripts lacking a **poly A** tail are unstable and are rapidly degraded. The **poly A** signal utilized in an expression vector may be "heterologous" or "endogenous." An endogenous **poly A** signal is one that is found naturally at the 3' end of the coding region of a given gene in the genome. A heterologous **poly A** signal is one which is one which is isolated from one gene and placed 3' of another gene. A commonly used heterologous **poly A** signal is the SV40 **poly A** signal. The SV40 **poly A** signal is contained on a 237 bp Bam HI/Bcl I restriction fragment and directs both termination and **polyadenylation** (J. Sambrook et al., supra, at 16.6-16.7).

DETD . . . FIG. 1). After deleting the regulatory elements including the NF-kB, Splbinding sites, and/or the TATA box, and inserting a minimal **cytomegalovirus** enhancer element, delayed replication kinetics has been observed in some CD4+ human lymphoid cell lines (See e.g., L. -J. Chang. . .

DETD It was also found that several LTR deletion mutants containing a **cytomegalovirus** enhancer element were capable of attenuating HIV-1 (i.e., the mutants were capable of infecting human lymphocytes with reduced cytopathic effects. . . was not markedly affected by these mutations. By mutating the tat gene, it was also found that the recombinant LTRs (**CMV**-**IE**-HIV-LTR) exhibited increased basal levels of promoter activity which could support virus replication without Tat (L. -J. Chang, and C. Zhang,. . .

DETD LTR mutants with kB/Spl or Sp1 deletion and **CMV**-**IE** enhancer/promoter insertion have been shown to replicate with delayed kinetics in human lymphocyte culture, including primary PBLs (peripheral blood lymphocytes). . .

DETD . . . were generated using the LTR mutant constructs which exhibited enhanced transcriptional activity after inserting heterologous enhancer elements. The recombinant LTR (**CMV**-**IE**-HIV-LTR), which has been shown to exhibit increased basal level of promoter activity, can support HIV-1 replication without Tat (L. -J.. . .

DETD . . . present invention, it was determined that the tat-C mutant is more defective than the tat-A and -B mutants, and the dl.Sp1/**CMV** tat-B double mutant is more defective than the dl.Sp1/**CMV** LTR mutant or the dl.Sp1/**CMV** tat-A double mutant reported previously (L. -J. Chang and C. Zhang, Virol., 211:157-169 [1995]). The dl.Sp1/**CMV** tat-B double mutant infects human lymphoid cell lines with delayed kinetics and exhibited reduced cytopathic effects.

DETD . . . PBLs poorly and replicated in primary macrophage culture with reduced kinetics. Based on these results, these already attenuated HIV-1 constructs, dl.Sp1/**CMV** tat-B and dl.Sp1/**CMV** tat-C, were chosen for HIV vector development.

DETD . . . LTR/tat mutants were further characterized in hunan lymphoid cell culture. The tat-A or tat-B LTR double mutants (Sp1 deleted and **CMV**-**IE** enhancer inserted) infected human MT4 cells with slightly reduced cytopathic effects. Further, these mutants exhibited delayed

replication kinetics when compared with wild type HIV-1. On the other hand, when cells were infected with the tat-C LTR mutant (Sp1/**CMV** mutant), the cytopathic effect was not so apparent and interestingly, the infected culture recovered rapidly and a persistent infection was.

. .

DETD . . . HIV-1 Infected Cultures

Cell Line/Virus	% Viability (±5%)	Doubling Time (±2 hrs)
MT4/(mock)	88	40
MT4/WT (acute)	0	--[a]
MT4/tat-A (dl.Sp1/**CMV**)	0	--
MT4/tat-B (Dl.Sp1/**CMV**)	0	--
MT4/tat-C (chr.1)	97	35
MT4/tat-C (chr.2)	86	32
AA2/WT (chr.)	73	n.d.[b]
Molt3/WT (chr.)	80	n.d.

[a] "--," No. . .

DETD . . . of bases). The nef-A sequence is the same as the wild-type sequence for the sequence shown starting at base 9001 (**ie.**, SEQ ID NO:6 represents the sequences for both wild-type and nef-A).

DETD . . . splice sequences, the entire gag-pol-env, vif, vpr, vpu, tat, and rev genes, a selectable gpt marker gene, and an SV40 **polyadenylation** signal as shown in FIG. 5, was cloned.

DETD Five additional HP constructs were also made ("pHP-VSVG," lipHP-**CMV**," "pHP-EF," "pHP-CMVdel.TAR/SD," and "pHP-**CMV**-EF1α-intron"), each with additional changes (See, FIG. 7). pHP-VSVG was derived from pHP-1, with the HIV-1 env gene being replaced by the VSV-G gene and containing either wild-type (pHP-NVSV-G) or mutated (pHP-VSV-G) vpr and tat genes. pHP-**CMV** was derived from pHP-1, with the promoter being replaced by the **cytomegalovirus immediate early promoter** (**CMV-IE**) and the tat, rev, env, vpr and vpu genes deleted. pHP-CMVdel.TAR/SD was derived from pHP-**CMV**, with the TAR and RSV RD deleted. pHP-**CMV**-EF1α-intron was derived from pHP-CMVdel.TAR/SD, with an insertion of the EF1α-intron between the promoter and the Gag AUG. pHP-EF was derived from pHP-**CMV**, by replacing the **CMV-IE** promoter and the synthetic SD site with the human elongation factor 1α (EF1α) enhancer plus intron. The TAR sequence was. . . transduction efficiency in nondividing culture. In other experiments, the intron-containing EF1α was shown to be a stronger promoter than the **CMV-IE** promoter.

DETD . . . of Gag-Pol (e.g., pHP-1, pHP-1del, and pHP-VSVG), as well as vectors that do not express detectable amounts of Gag-Pol (e.g., pHP-**CMV** and its derivatives).

DETD . . . ψ signals were cloned into the pTVψ vector as shown in FIG. 8, which is comprised of two recombinant LTRs ("dl.kB-**CMV**/HIV-TAR"), the PBS and 5' leader sequences, an SV40-driven neo resistance gene, and the 3' PPT.

DETD . . . an additional gag sequence and an RRE element, were cloned into pTVψ140. One such example is shown in FIG. 9A (pTVψ+**CMV**-nlacZ-hyg). Again, the pTVψ+ was not packaged efficiently, indicating the splice donor site and Gag AUG mutations in pTVψ100 and pTVψ140.

. .

DETD Three additional pTVA vectors were also constructed, each containing a different reporter gene: **CMV**-GFP (green fluorescent protein, pTVΔ**CMV**-GFP), **CMV**-nlacZ (pTVΔ**CMV**-nlacZ) and **CMV**-nlacZ-hyg (pTVΔ**CMV**-nlacZ-hyg), as illustrated in FIG. 8 (See, FIG. 8, constructs 5 and 6, as well as FIG. 9B). The production of VSV-G pseudotyped vector was tested with pTVΔ**CMV**-nlacZ. TE671 cells transduced with the VSV-G pseudotyped pTVΔ**CMV**-nlacZ vector stained strongly by X-gal and exhibited nuclear β-galactosidase activity. The pTVΔ**CMV**-nlacz-hyg and pTVΔ**CMV**-GFP did not express the reporter genes efficiently, whereas pTVΔ**CMV**-nlacZ did. These transducing vectors were further characterized using dividing

and nondividing tissue culture models and a small animal model.

DETD . . . (scanning electron microscope); TLC (thin layer chromatography); tRNA (transfer RNA); nt (nucleotide); VRC (vanadyl ribonucleoside complex); RNase (ribonuclease); DNase (deoxyribonuclease); **poly A** (polyriboadenylic acid); PBS (phosphate buffered saline); OD (optical density); HEPES (N-[2-Hydroxyethyl]piperazine-N-[2-ethanesulfonic acid]); HBS (HEPES buffered saline); SDS (sodium dodecyl sulfate);. . .

DETD . . . derived from RSV, the entire gag-pol-env,vif, vpr, vpu, tat, and rev genes, a selectable gpt marker gene, and an SV40 **polyadenylation** signal as shown in FIG. 5 was developed.

DETD . . . by recombination. Thus, pHP-1 provides an excellent HIV DNA vector. pHP-1 was constructed as follows. First, the Tat-responsive enhancer promoter **CMV**-TATA-TAR fragment (approximately 400 bp) was isolated from dl.kB/Sp1-**CMV**-TATA-TAR HIV (Chang et al., J. Virol. 67:743 [1993]) by BbrpI-HindIII digestion, and cloned into EcoRV-BamHI digested pSP72 (Promega) via a linker providing HindIII and BamHI cohesive sites which contains a modified gag AUG with **Kozak** translation initiation context and a major splice donor site of Rous sarcoma virus. This linker was formed by annealing the following oligonucleotides: 5'-AGCTTGGTCGCCCGGTGGATCAAGACCGGTAGCCGTCATAAAGGTGAT TTCGTCG-3' (SEQ ID NO:9) and 5'-GATCCGACGAAATCACCTTTATGACG GCTACCGGTCTTGATCCACCGGGCGACCA-3' (SEQ ID NO:10). This first subclone was called pSP-**CMV**-TAR-SD.

DETD . . . [SEQ ID NO:12]). The PCR product was digested with BamHI-SphI (.about.660 bp) and this fragment was ligated with BamHI-SphI digested pSP-**CMV**-TAR-SD to obtain pSP-**CMV**-TAR-SD-dl.gag.

DETD Next, the **poly-A** minus subclone pHP-dl.pA was constructed by ligating the following three fragments: a 1112 bp HpaI-SphI fragment isolated from pSP-**CMV**-TAR-SD-dl.gag (contains the promoter-TAR-SD-dl.gag), a 7922 bp SphI-XhoI fragment (dl.gag-pol-env-gpt) of pNLgpt, and a plasmid vector backbone provided by EcoRV-XhoI digested. . .

DETD Lastly, pHP-1 was made by the following ligation: NotI-XhoI (9059 bp) of pHP-dl.pA containing dl.**CMV**-TATA-TAR-SD-gag-pol-env-gpt, a 422 bp **poly-A** site from XhoI-PstI digested pREP9 (Invitrogen), and NotI-PstI digested pBS-KS(-). The sequence of pHP-1 (12,494 kb) is provided in SEQ. . .

DETD As described in more detail below, five other HP constructs were made, pHP-VSVG, three pHP-**CMV** derivatives, and pHP-EF, each with additional changes (See, FIG. 7). pHP-VSVG was derived from pHP-1, with the HIV-1 env gene. . . by the VSV-G gene, and with wild-type vpr and tat, or the vpr and tat genes mutated by site-specific mutagenesis. pHP-**CMV** was derived from pHP-1 with the promoter being replaced by the **cytomegalovirus immediate early promoter** (**CMV-IE**) and the tat, rev, env, vpr and vpu deleted. pHP-CMVdel.TAR/SD was derived from pHP-**CMV**, with the TAR and RSV RD deleted. pHP-**CMV**-EF1α-intron was derived from pHP-CMVdel.TAR/SD, with an insertion of the EF1α-intron between the promoter and the Gag AUG. pHP-EF was derived from pHP-**CMV** by replacing the **CMV-IE** promoter and the synthetic SD site with the human elongation factor 1α (EF1α) enhancer plus intron. It also contains an. . . the vector transduction efficiency in non-dividing cultures. The intron-containing EF1a has been shown to be a stronger promoter than the **CMV-IE** promoter. These constructs were tested for their expression of HIV-1 proteins. pHP-VSVG did not express HIV-1 proteins unless the Tat. . .

DETD Both packaging constructs (i.e., pHP-1 and pHP-VSVG) used a recombinant **CMV**/HIV-LTR as promoter and a synthetic major splice donor site. No sequence homology was observed with the HIV-1 genome between TAR. . .

DETD These experiments showed that pHP-**CMV** and pHP-EF do not express Gag-Pol protiens at high efficiencies, indicating that the pHP-1-derived vectors have important viral sequences that. . .

DETD . . . vpr and tat genes. It was constructed by combining the following four pieces of DNA fragments: 1) the recombinant LTR (dl.kB/Sp1-**CMV**-TATA-HIV-TAR) gag-pol from NotI to EcoRI fragment of pHP-1; 2) a fragment from HIV-1 with deletion in the C-terminal of Vpr. . .

DETD pHP-**CMV**:

DETD This clone was derived from pHP-1, with the 5' recombinant LTR replaced by a **CMV-IE** enhancer-promoter and the entire env, tat, vpu, rev, vpr, nef deleted, but with the vif gene remaining intact. This clone. . .

DETD pHP-**CMV**-del.TAR/SD:

DETD This clone is the same as pHP-**CMV** except that the 5' TAR and splice donor site are deleted. This construction was made by ligating the following two fragments: 1) a 702 bp fragment of MluI-BamHI digested pcDNA3.1Zeo(+) containing the **CMV** enhancer; and 2) the vector containing MluI-BamHI digested pHP-**CMV** which has deleted TAR and contains the RSV splice donor site.

DETD pHP-**CMV**-EF1α-intron.

DETD This clone is similar to pHP-**CMV**-del.TAR/SD but with an intron from human EF-1a gene inserted between the **CMV** promoter and the gag AUG. It was made by ligating the following three DNA fragments: 1) pHP-1 BamHI-EcoRI fragment containing. . . gag-pol and vif; 2) the MluI-EcoRI of pcDNAZeonlacZ-RRE containing the vector backbone of pcDNA3.1Zeo(+), HIV-1 RRE and part of the **CMV** promoter; and 3) the rest of the **CMV** enhancer promoter was obtained from BamHI-MluI digested pcDNAZeoHGHP2EF, a pcDNAZeo3.1(+) vector containing EF1α intron and the human growth hormone gene.. . .

DETD Four additional packaging vectors, pHP-**CMV** derivatives, and pHP-EF, were constructed as shown in FIG. 7. The heterologous enhancer/promoters in these vectors may express high levels. . . of GFP is much improved when an intron sequence was inserted in front of the GFP gene. All of the pHP-**CMV** derivatives were tested, and found to be inefficient in synthesizing HIV proteins, indicating that the pHP-1 and pHP-VSVG derivatives are. . .

DETD . . . or without a splice donor site, both obtainable from the pHP vectors. The 3' LTR is replaced by the SV40 **polyadenylation** signal. The nef and env genes are both deleted from the vector. The expression of vpx is included in the. . .

DETD . . . sequence. Sequences in gag-pol and env genes are deleted and the major SD and the gag AUG are mutated. A **CMV**-driven reporter gene cassette such as the **CMV-IE**-nlacZ-IRES-hyg from the pTVA-nlacZ-hyg vector is inserted in the nef ORF of the HIV-2 and the SIV vectors. The 3' LTR. . .

DETD Internal **CMV-IE** in pTVΔCMVnlacZ Promoter Exhibits Higher Promoter Activity Than Native **CMV-IE**

DETD In this Example, the expression of the reporter lacZ gene from the pTV-ΔCMVnlacZ was compared with pcDNAnlacZ (i.e., **CMV-IE** promoter-driven), 48 hours after transfection of TE671 cells. TE671 cells were transfected with 5 μg of pcDNA3-nlacZ or pTVΔCMVnlacZ, as. . .

DETD . . . sequences near the 5' end of the PPT of HIV-1, the product was then ligated with a SalI-KpnI fragment containing **CMV**-nlacZ sequence from pcDNAzeo-nlacZ. pcDNAzeo-nlacZ was generated by inserting nlacZ of pSP72nlacZ into pcDNA3.1zeo(+).

DETD TABLE 3

Production of High-Titer HIV-1 Derived Vectors

Packaging Construct	Pseudotyped Envelope	Transducing Vector	Additional Genes	RT (cpm/μl)	Titer (cfu/ml)
pNL4-3	pHEF-VSVG	pTVΔ**CMV**-nlacZ		1.1×10^5	7.9×10^4
pNL-4-3	pHEF-VSVG	pTVψ**CMV**-nlacZ-hyg-dl.SmaI		7.9×10^4	24
pHP-1	pHEF-VSVG	pTVΔ**CMV**-nlacZ	pCEP-tat	3.7×10^4	2.5×10^5
pHP-1	pHEF-VSVG	pTVψ**CMV**-nlacZ	pCEP-	3.1×10^4	100

VSVG nlacZ hyg tat
dl.SmaI
pHP-2dl.2 pHEF- pTVΔ**CMV** pCEP- 3.9 × 10^4 1.7 × 10^5
VSVG nlacZ tat
pHP-1dl.2 pHEF- pTVψ**CMV**- pCEP- 3.6 × 10^4 90
VSVG nlacZ-hyg- tat
dl.SmaI

DETD . . . 4
Detection of Replication-Competent HIV (RCV)

Packaging Construct	Pseudotyped Envelope	Trans-ducing Vector	Addi-tional Genes	Days After Co-Culture 8	28	60#
pNL4-3 (Control)	pHEF-VSVG	pTVΔ**CMV**-nlacZ		++++	+*	+++
pHP-1	pHEF-VSVG	pTVΔ**CMV**-nlacZ	pCEP-tat	++	+++	+++
pHP-1dl.2	pHEF-VSVG	pTVΔ**CMV**-nlacZ	pCEP-tat	-	-	-
pHP-1dl.28	pHEF-VSVG	pTVΔ**CMV**-nlacZ	pCEP-tat	-	-	-

*Results of rapid cell death and loss of MT4 cells.
`+ to ++++`, approximately 10. . .
DETD . . . (BAS1000). The results are shown in FIG. 14: Lane 1, control MT4; lane 2 &3, MT4 chronically infected with dl.Sp1 **CMV** tat-C; lane 4, MT4 acutely infected with WT HIV-1; lane 5, C8166 chronically infected with WT HIV-1; lane 6, MT4 chronically infected with dl.Sp1 **CMV** tat-B; lane 7, AA2 chronically infected with dl.Sp1 **CMV** tat-C.
CLM What is claimed is:
. . . 15. The packaging vector of claim 1 in which the 5'LTR is a chimera of a lentivirus LTR and a **CMV** enhancer/promoter.

=> d his

(FILE 'HOME' ENTERED AT 15:58:13 ON 30 APR 2004)

FILE 'USPATFULL' ENTERED AT 15:59:07 ON 30 APR 2004
E CHANG GWONG JEN/IN
L1 1 S E4

FILE 'WPIDS' ENTERED AT 16:00:09 ON 30 APR 2004
E CHANG G J/IN
L2 106 S E3
L3 2 S L2 AND FLAVIVIR?

FILE 'MEDLINE' ENTERED AT 16:01:13 ON 30 APR 2004
E CHANG G J/AU
L4 49 S E3
L5 29 S L4 AND (FLAVIVIR? OR YELLOW OR DENGUE OR ENCEPHALITIS)
L6 9 S L5 AND (PRM OR E OR PRM/M OR PRM/E)

FILE 'USPATFULL' ENTERED AT 16:06:37 ON 30 APR 2004
L7 3470 S (FLAVIVIR? OR YELLOW FEVER VIR? OR DENGUE OR ENCEPHALITIS VIR
L8 79 S L7 AND (PRM OR PREMEMBRANE)
L9 79 S L8 AND (E OR ENVELOPE)
L10 79 S L9 AND (M OR MEMBRANE)
L11 43 S L10 AND (SIGNAL SEQUENCE)
L12 5 S L11 AND KOZAK
L13 38 S L11 NOT L12
L14 15 S L13 AND AY<1999
L15 805 S L7 AND (KOZAK OR RIBOSOME BINDING SITE)
L16 2 S L15 AND KOZAK/CLM
L17 0 S L15 AND (KOZAK RIBOSOME BINDING SITE? OR KOZAK CONCENSUS SEQU

```
L18          710 S L15 AND (CMV OR CYTOMEGALOVIRUS)
L19          191 S L18 AND (IE OR IMMEDIATE-EARLY PROMOTER?)
L20           30 SS L19 AND (CMV/CLM OR CYTOMEGALOVIRUS/CLM)
L21            2 S L20 AND (IE/CLM OR IMMEDIATE-EARLY/CLM)
L22           28 S L20 NOT L21
L23           10 S L20 AND AY<1999
L24          743 S L15 AND (POLYADENYLATION OR POLY-A)
L25          423 S L24 AND (POLYADENYLATION (5W) TERMINAT?)
L26            2 S L25 AND L23

=> e konishi e/in
E1             2     KONISHI CHIZUKO/IN
E2             2     KONISHI DAISUKE/IN
E3             0 --> KONISHI E/IN
E4             1     KONISHI EIICHIRO/IN
E5             1     KONISHI FUMIKO/IN
E6             4     KONISHI FUMIYA/IN
E7             8     KONISHI GAKU/IN
E8             2     KONISHI GIICHI/IN
E9             2     KONISHI GREGORY A/IN
E10            4     KONISHI HAJIME/IN
E11            2     KONISHI HARUKO/IN
E12            9     KONISHI HARUO/IN

=> s e4
L27            1 "KONISHI EIICHIRO"/IN

=> d 127,ti

L27  ANSWER 1 OF 1  USPATFULL on STN
TI     Hydraulic hardening material and method of manufacturing the same


=> e kozak m/in
E1             1     KOZAK LARRY S/IN
E2             1     KOZAK LINDA K/IN
E3             0 --> KOZAK M/IN
E4             3     KOZAK MAREK Z/IN
E5             1     KOZAK MARIAN/IN
E6             2     KOZAK MARK/IN
E7             1     KOZAK MARK W/IN
E8             1     KOZAK MARY B/IN
E9             2     KOZAK MICHAEL/IN
E10            1     KOZAK MICHAEL J/IN
E11            1     KOZAK MICHAEL L/IN
E12            4     KOZAK MILAN/IN

=> s e5
L28            1 "KOZAK MARIAN"/IN

=> d 128,ti

L28  ANSWER 1 OF 1  USPATFULL on STN
TI     Dynamic automatic gain control circuit employing kalman filtering


=> file medline
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                      ENTRY     SESSION
FULL ESTIMATED COST                                  189.87      215.49

FILE 'MEDLINE' ENTERED AT 16:45:49 ON 30 APR 2004

 FILE LAST UPDATED: 29 APR 2004 (20040429/UP).  FILE COVERS 1951 TO DATE.

 On February 29, 2004, the 2004 MeSH terms were loaded.  See HELP RLOAD
```

for details. OLDMEDLINE now back to 1951.

MEDLINE thesauri in the /CN, /CT, and /MN fields incorporate the MeSH 2004 vocabulary. See http://www.nlm.nih.gov/mesh/ and http://www.nlm.nih.gov/pubs/techbull/nd03/nd03_mesh.html for a description of changes.

This file contains CAS Registry Numbers for easy and accurate substance identification.

```
=> e konishi e/au
E1            2     KONISHI DE TOFFOLI G/AU
E2            1     KONISHI DE TOFFOLI J/AU
E3           96 --> KONISHI E/AU
E4            1     KONISHI E J/AU
E5            5     KONISHI EIICHI/AU
E6            9     KONISHI EIJI/AU
E7            2     KONISHI EISAKU/AU
E8            4     KONISHI EMIKO/AU
E9          174     KONISHI F/AU
E10           3     KONISHI FUMIKO/AU
E11          29     KONISHI FUMIO/AU
E12           2     KONISHI FUSAKO/AU

=> s e3-e5
             96 "KONISHI E"/AU
              1 "KONISHI E J"/AU
              5 "KONISHI EIICHI"/AU
L29         102 ("KONISHI E"/AU OR "KONISHI E J"/AU OR "KONISHI EIICHI"/AU)

=> s l29 and (prM or premembrane)
            544 PRM
             87 PREMEMBRANE
L30          17 L29 AND (PRM OR PREMEMBRANE)

=> s l30 and (E or envelope or env)
         628030 E
          34155 ENVELOPE
           7108 ENV
L31           17 L30 AND (E OR ENVELOPE OR ENV)

=> d l31,cbib,ab,1-17

L31  ANSWER 1 OF 17     MEDLINE on STN
2001180918.    PubMed ID: 11160724.   Generation and characterization of a
     mammalian cell line continuously expressing Japanese encephalitis virus
     subviral particles. Konishi E; Fujii A; Mason P W. (Department of Health
     Sciences, Kobe University School of Medicine, Kobe 654-0142, Japan..
     ekon@ams.kobe-u.ac.jp) . Journal of virology, (2001 Mar) 75 (5) 2204-12.
     Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States.
     Language: English.
AB   We have generated a cell line (F cells) producing a secreted form of
     Japanese encephalitis virus (JEV) subviral particle (extracellular
     particles [EPs]) that contains the JEV envelope glycoprotein (E) and a
     precursor (prM) of the virion membrane protein (M). The F cells were
     engineered to synthesize these JEV products from a cDNA encoding a mutated
     (furin proteinase resistant) form of prM, since stable cell lines
     expressing E and the authentic form of prM could not be obtained, due
     (in part) to the cell-fusing ability of EPs containing E and M. Our
     biochemical alteration of the prM protein was critical for the
     successful production of EP-producing cell lines. EPs produced by F cells
     share the biochemical properties of empty viral particles produced by
     JEV-infected cells, except that the F-cell EPs lack hemagglutinating
     activity and M. F-cell EPs were recognized by a panel of monoclonal
     antibodies to E, and EPs were shown to be useful as vaccine candidates
     in mice and as diagnostic reagents in evaluating human immune responses to
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JE vaccination. The amounts of E antigen released into the culture fluid of F cells were similar to those found in virion fractions of JEV-infected cell culture fluids or JEV-infected weanling mouse brains (the current source of antigen used to produce human vaccines for JE). Thus, the F-cell line would appear to be a useful source of antigen for JE vaccines and diagnostics.

L31 ANSWER 2 OF 17 MEDLINE on STN

2000254536. PubMed ID: 10795519. Definition of an epitope on Japanese encephalitis virus (JEV) **envelope** protein recognized by JEV-specific murine CD8+ cytotoxic T lymphocytes. Takada K; Masaki H; **Konishi E**; Takahashi M; Kurane I. (Department of Neurology, Kinki University School of Medicine, Osakasayama, Japan.) Archives of virology, (2000) 145 (3) 523-34. Journal code: 7506870. ISSN: 0304-8608. Pub. country: Austria. Language: English.

AB We defined an epitope on the Japanese encephalitis virus (JEV) **envelope** (**E**) protein recognized by CD8+ cytotoxic T lymphocytes (CTLs). CTLs induced in JEV-infected BALB/c (H-2d) mice recognized **E** and/or **premembrane** (**PrM**) proteins, while CTLs in C57BL/6J (H-2b) and C3H/HeJ (H-2k) mice did not. JEV-specific CTLs had a phenotype of CD3+ CD4- CD8+. Twenty-four 9-amino acid (a.a.) peptides, which had binding motifs for H-2Kd, H-2Ld or H-2Dd, were synthesized according to the amino acid sequences of **PrM** and **E** proteins. CTLs induced by JEV infection recognized only the peptide K-3. Immunization of BALB/c mice with only a group of peptides including K-3 induced CTLs which recognized the homologous K-3 peptide, while immunization with other peptides did not. The peptide K-3 had a binding motif for H-2Kd. This is consistent with the finding that JEV-specific CTLs in BALB/c mice was H-2Kd-restricted. These results indicate that the epitope recognized by CTLs in BALB/c mice is located between a.a. 60 and 68 on the **E** protein, corresponding to an a.a. sequence of CYHASVTDI.

L31 ANSWER 3 OF 17 MEDLINE on STN

2000149220. PubMed ID: 10683326. Japanese encephalitis DNA vaccine candidates expressing **premembrane** and **envelope** genes induce virus-specific memory B cells and long-lasting antibodies in swine. **Konishi E**; Yamaoka M; Kurane I; Mason P W. (Department of Health Sciences, Kobe University School of Medicine, Kobe, 654-0142, Japan.. ekon@ams.kobeu.ac.jp) . Virology, (2000 Mar 1) 268 (1) 49-55. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.

AB Swine are an important amplifier of Japanese encephalitis (JE) virus in the paradomestic environment. In this study, two JE DNA vaccine candidates were evaluated for immunogenicity in swine. Both vaccine plasmids encode a cassette consisting of the signal of **premembrane** (**prM**), **prM**, and **envelope** (**E**) coding regions of JE virus. One plasmid, designated pcJEME, is based on a commercial vector (pcDNA3), whereas the other plasmid, designated pNJEME, is based on a vector (pNGVL4a) designed to address some of the safety concerns of DNA vaccine use. No differences were detected in the immunogenicity of these two plasmids in mice or swine. Swine immunized with the DNA vaccines at a dose of 100 to 450 microgram at an interval of 3 weeks developed neutralizing and hemagglutination-inhibitory (HAI) antibody titers of 1:40 to 1:160 at 1 week after the second immunization. However, swine administered two doses of a commercial JE vaccine (formalin-inactivated virus preparation; JEVAX-A) developed low (1:10) or undetectable antibody responses after their boost. Interestingly, serum antibody titers elicited by DNA vaccines in swine were higher than those detected in mice. Eight days after boosting with viral antigen (JEVAX-A) to detect an anamnestic response, swine immunized two times with the DNA vaccine showed a >100-fold elevation in HAI titer, indicating a strong recall of antibody response. Swine maintained detectable levels of HAI antibody for at least 245 days after two immunizations with a DNA vaccine. These results indicate that these DNA vaccines are able to induce virus-specific memory B cells and long-lasting antibodies in swine, which were of higher levels than those obtained with a commercial formalin-inactivated JE vaccine.

L31 ANSWER 4 OF 17 MEDLINE on STN

2000059995. PubMed ID: 10590335. A DNA vaccine expressing dengue type 2 virus **premembrane** and **envelope** genes induces neutralizing antibody and memory B cells in mice. **Konishi E**; Yamaoka M; Kurane I; Mason P W. (Department of Health Sciences, Kobe University School of Medicine, 7-10-2 Tomogaoka, Suma-ku, Kobe, Japan.. ekon@ams.kobe-u.ac.jp) . Vaccine, (2000 Jan 6) 18 (11-12) 1133-9. Journal code: 8406899. ISSN: 0264-410X. Pub. country: ENGLAND: United Kingdom. Language: English.

AB A dengue DNA vaccine candidate was developed and evaluated for immunogenicity in mice. The vaccine, designated pcD2ME, is a pcDNA3-based plasmid encoding the signal sequence of **premembrane** (**prM**), **prM** and **envelope** (**E**) genes of the New Guinea C strain of dengue type 2 virus. CHO-K1 cells transfected with pcD2ME expressed **prM** and **E** as determined by immunochemical staining with monoclonal antibodies. BALB/c mice inoculated intramuscularly with 100 microg of pcD2ME two or three times at an interval of 2 weeks developed a low level of neutralizing antibody (1:10 at a 90% plaque reduction). Immunization twice with 10 microg or 1 microg of pcD2ME or three times with 100 microg of pcDNA3 did not induce detectable levels of neutralizing antibody. Mice immunized two or three times with 100 microg of pcD2ME raised neutralizing antibody titers to 1:40 or greater on days 4 and 8 after challenge with 3x10(5) plaque forming units (PFU) of the New Guinea C strain of dengue type 2 virus, showing strong anamnestic responses to the challenge. In contrast, mice immunized two or three times with 100 microg of pcDNA3 developed no detectable neutralizing antibody on days 4 and 8 after challenge. These results indicate that immunization with pcD2ME induces neutralizing antibody and dengue type 2 virus-responsive memory B cells in mice.

L31 ANSWER 5 OF 17 MEDLINE on STN

1999292845. PubMed ID: 10364301. The anamnestic neutralizing antibody response is critical for protection of mice from challenge following vaccination with a plasmid encoding the Japanese encephalitis virus **premembrane** and **envelope** genes. **Konishi E**; Yamaoka M; Khin-Sane-Win; Kurane I; Takada K; Mason P W. (Department of Health Sciences, Kobe University School of Medicine, Kobe 654-0142, Japan.. ekon@ams.kobe-u.ac.jp) . Journal of virology, (1999 Jul) 73 (7) 5527-34. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB For Japanese encephalitis (JE), we previously reported that recombinant vaccine-induced protection from disease does not prevent challenge virus replication in mice. Moreover, DNA vaccines for JE can provide protection from high challenge doses in the absence of detectable prechallenge neutralizing antibodies. In the present study, we evaluated the role of postchallenge immune responses in determining the outcome of JE virus infection, using mice immunized with a plasmid, pcDNA3JEME, encoding the JE virus **premembrane** (**prM**) and **envelope** (**E**) coding regions. In the first experiment, 10 mice were vaccinated once (five animals) or twice (remainder) with 100 micrograms of pcDNA3JEME. All of these mice showed low (6 of 10) or undetectable (4 of 10) levels of neutralizing antibodies. Interestingly, eight of these animals showed a rapid rise in neutralizing antibody following challenge with 10,000 50% lethal doses of JE virus and survived for 21 days, whereas only one of the two remaining animals survived. No unimmunized animals exhibited a rise of neutralizing antibody or survived challenge. Levels of JE virus-specific immunoglobulin M class antibodies were elevated following challenge in half of the unimmunized mice and in the single pcDNA3JEME-immunized mouse that died. In the second experiment, JE virus-specific primary cytotoxic T-lymphocyte (CTL) activity was detected in BALB/c mice immunized once with 100 micrograms of pcDNA3JEME 4 days after challenge, indicating a strong postchallenge recall of CTLs. In the third experiment, evaluation of induction of CTLs and antibody activity by plasmids containing portions of the **prM/E** cassette demonstrated that induction of CTL responses alone were not sufficient to prevent death. Finally, we showed that antibody obtained from pcDNA3JEME-immunized mice 4 days following challenge could partially protect recipient mice from lethal challenge.

taken together, these results indicate that neutralizing antibody produced following challenge provides the critical protective component in pcDNA3JEME-vaccinated mice.

L31 ANSWER 6 OF 17 MEDLINE on STN

1998291412. PubMed ID: 9627942. Induction of Japanese encephalitis virus-specific cytotoxic T lymphocytes in humans by poxvirus-based JE vaccine candidates. **Konishi E**; Kurane I; Mason P W; Shope R E; Kanesa-Thasan N; Smucny J J; Hoke C H Jr; Ennis F A. (Department of Medical Zoology, Kobe University School of Medicine, Japan.. ekon@ams.kobe-u.ac.jp) . Vaccine, (1998 May) 16 (8) 842-9. Journal code: 8406899. ISSN: 0264-410X. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Poxvirus-based recombinant Japanese encephalitis (JE) vaccine candidates, NYVAC-JEV and ALVAC-JEV, were examined for their ability to induce JE virus-specific cytotoxic T lymphocytes (CTLs) in a phase I clinical trial. These vaccine candidates encoded the JE virus **premembrane** (**prM**), **envelope** (**E**) and non-structural 1 (NS1) proteins. The volunteers received subcutaneous inoculations with each of these candidates on days 0 and 28, and blood was drawn 2 days before vaccination and on day 58. Anti-**E** and anti-NS1 antibodies were elicited in most vaccinees inoculated with NYVAC-JEV and in some vaccinees inoculated with ALVAC-JEV. Peripheral blood mononuclear cells (PBMCs) obtained from approximately one half of vaccines showed positive proliferation in response to stimulation with live JE virus. Cytotoxic assays demonstrated the presence of JE virus-specific CTLs in in vitro-stimulated PBMCs obtained from two NYVAC-JEV and two ALVAC-JEV vaccinees. Cell depletion tests using PBMCs from one NYVAC-JEV recipient indicated that the phenotype of CTLs was CD8+CD4-.

L31 ANSWER 7 OF 17 MEDLINE on STN

1998241731. PubMed ID: 9573260. Induction of protective immunity against Japanese encephalitis in mice by immunization with a plasmid encoding Japanese encephalitis virus **premembrane** and **envelope** genes. **Konishi E**; Yamaoka M; Khin-Sane-Win; Kurane I; Mason P W. (Department of Medical Zoology, Kobe University School of Medicine, Kobe 650, Japan.. ekon@ams.kobe-u.ac.jp) . Journal of virology, (1998 Jun) 72 (6) 4925-30. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB A DNA vaccine plasmid containing the Japanese encephalitis (JE) virus **premembrane** (**prM**) and **envelope** (**E**) genes (designated pcDNA3JEME) was evaluated for immunogenicity and protective efficacy in mice. Two immunizations of 4-week-old female ICR mice with pcDNA3JEME by intramuscular or intradermal injections at a dose of 10 or 100 microg per mouse elicited neutralizing (NEUT) antibodies at titers of 1:10 to 1:20 (90% plaque reduction), and all immunized mice survived a challenge with 10,000 50% lethal doses of the P3 strain of JE virus. A single immunization with 100 microg of pcDNA3JEME did not elicit detectable NEUT antibodies but induced protective immunity. Spleen cells obtained from BALB/c mice immunized once with 10 or 100 microg of pcDNA3JEME contained JE virus-specific memory cytotoxic T lymphocytes (CTLs). BALB/c mice maintained detectable levels of memory B cells and CTLs for at least 6 months after one immunization with pcDNA3JEME at a dose of 100 microg. The CTLs induced in BALB/c mice immunized twice with 100 microg of pcDNA3JEME were CD8 positive and recognized mainly the **envelope** protein. These results indicate that pcDNA3JEME has the ability to induce a protective immune response which includes JE virus-specific antibodies and CTLs.

L31 ANSWER 8 OF 17 MEDLINE on STN

97227581. PubMed ID: 9139487. Particulate vaccine candidate for Japanese encephalitis induces long-lasting virus-specific memory T lymphocytes in mice. **Konishi E**; Win K S; Kurane I; Mason P W; Shope R E; Ennis F A. (Department of Medical Zoology, Kobe University School of Medicine, Japan.) Vaccine, (1997 Feb) 15 (3) 281-6. Journal code: 8406899. ISSN: 0264-410X. Pub. country: ENGLAND: United Kingdom. Language: English.

AB We previously reported that extracellular particles (EPs) composed of **premembrane** (**prM**) and **envelope** (**E**) proteins were released from cells infected with recombinant vaccinia viruses encoding Japanese encephalitis (JE) virus **prM** and **E** genes. In the present study, EPs were evaluated for induction of JE virus-specific antibody and specific T lymphocytes in mice. Six- to 8-week-old male Balb/c mice were inoculated intraperitoneally once or twice (at a 3-week interval) with purified EPs containing 1 microgram of **E** without adjuvant. Neutralizing antibody was detected and spleen cells proliferated against JE viral antigen 3 weeks after the second immunization with EPs. Neutralizing antibody and JE virus-specific T lymphocytes were also detected 10 months after immunization with EPs containing 2 micrograms of **E**. Spleen cells obtained from EP-immunized mice and stimulated in vitro with live JE virus, expressed JE virus-specific cytotoxic activity. The cytotoxic activity was reduced by treatment with anti-CD3 antibody and complement. These results indicate that immunization with EPs induces long-lasting specific antibody and memory T cells in mice.

L31 ANSWER 9 OF 17 MEDLINE on STN

97170855. PubMed ID: 9018134. Poxvirus-based Japanese encephalitis vaccine candidates induce JE virus-specific CD8+ cytotoxic T lymphocytes in mice. **Konishi E**; Kurane I; Mason P W; Shope R E; Ennis F A. (Department of Medical Zoology, Kobe University School of Medicine, Chuo-ku, Japan.. ekon@icluna.kobe-u.ac.jp) . Virology, (1997 Jan 20) 227 (2) 353-60. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.

AB Recombinant Japanese encephalitis (JE) vaccine candidates based on a highly attenuated vaccinia virus (NYVAC-JEV) and a canarypox virus (ALVAC-JEV) were evaluated for their ability to induce specific antibodies and cytotoxic T lymphocytes (CTLs) in mice. Six- to eight-week-old male Balb/c mice that received one or two intraperitoneal inoculations with these JE vaccine candidates at a dose of 1 x 10(7) PFU per mouse produced neutralizing antibody and antibodies to the **envelope** (**E**) and nonstructural 1 (NS1) proteins as determined by radioimmunoprecipitation. Immunization with either of these vaccine candidates also induced JE virus-specific T lymphocytes that proliferated in response to stimulation with infectious virus and/or noninfectious viral antigens. Mice maintained detectable levels of neutralizing antibody and JE virus-specific memory T cells for at least 6 months after immunization with NYVAC-JEV and for 4 months after immunization with ALVAC-JEV. Cells induced to proliferate after stimulation with live virus contained specific CD8+ CTLs that lysed primary Balb/c mouse kidney cells infected with JE virus and P815 mastocytoma cells infected with a recombinant vaccinia virus expressing the **premembrane** (**prM**), **E**, and NS1 proteins. These CTLs also lysed P815 cells infected with vaccinia recombinants expressing **prM** and **E**, and those expressing **E** and NS1, but did not lyse P815 cells infected with a recombinant virus expressing only NS1, indicating that the CTLs mainly recognized **E**, but did not recognize NS1. These results demonstrate that both recombinant JE vaccines, NYVAC-JEV and ALVAC-JEV, induce JE virus-specific antibody and CTLs in mice.

L31 ANSWER 10 OF 17 MEDLINE on STN

96423113. PubMed ID: 8825714. Enzyme-linked immunosorbent assay using recombinant antigens for serodiagnosis of Japanese encephalitis. **Konishi E**; Mason P W; Shope R E. (Department of Medical Zoology, Kobe University School of Medicine, Japan.) Journal of medical virology, (1996 Jan) 48 (1) 76-9. Journal code: 7705876. ISSN: 0146-6615. Pub. country: United States. Language: English.

AB Recombinant Japanese encephalitis (JE) virus proteins were evaluated as antigens for serodiagnosis of JE using an enzyme-linked immunosorbent assay (ELISA). The **premembrane**/membrane (**prM**/M) and **envelope** (**E**) proteins of JE virus were expressed in HeLa cells infected with a recombinant vaccinia virus that encodes the JE virus **prM** and **E** genes and were released from cells in a particulate form. The particulate antigens were partially purified from culture fluid from the infected

cells by precipitation of particles with polyethylene glycol and then dissociated from the particles with 0.1% Triton X-100. This antigen preparation was used to evaluate one preimmune and two postvaccination sera from 20 volunteers given three inoculations of the commercial JE vaccine (Biken vaccine) by a conventional ELISA. The results from this assay correlated with neutralization data. The results of an IgM capture ELISA carried out with the recombinant antigen also correlated with the results of an existing IgM capture ELISA performed with JE virus-infected mouse brain, when tested with 29 serum and 13 cerebrospinal fluid samples from JE patients. These results indicated that recombinant JE virus antigens are useful for ELISA as an antigenically equivalent, highly productive, and safe alternative to authentic JE virus antigens.

L31 ANSWER 11 OF 17 MEDLINE on STN

96033014. PubMed ID: 7573713. Japanese encephalitis virus-specific proliferative responses of human peripheral blood T lymphocytes. **Konishi E**; Kurane I; Mason P W; Innis B L; Ennis F A. (Department of Medical Zoology, Kobe University School of Medicine, Japan.) American journal of tropical medicine and hygiene, (1995 Sep) 53 (3) 278-83. Journal code: 0370507. ISSN: 0002-9637. Pub. country: United States. Language: English.

AB The T lymphocytes play an important role in prevention and recovery from viral infections. To characterize T lymphocyte responses to Japanese encephalitis (JE) virus infections, we analyzed JE virus-specific T lymphocytes in peripheral blood mononuclear cells (PBMC) obtained from seven JE patients and 10 vaccinees who had received a formalin-inactivated, purified JE virus vaccine (Biken vaccine). These PBMC were examined for proliferative responses against live JE virus, a glutaraldehyde-fixed lysate of cells infected with JE virus, and extracellular particles (EPs; subviral membrane vesicles released from cells infected with recombinant vaccinia viruses encoding the JE virus **premembrane** and **envelope** proteins). Japanese encephalitis virus-specific T cell proliferation was demonstrated with PBMC from both patients and vaccinees after stimulation with infectious JE virus or the lysate of JE virus-infected cells. Proliferating PBMC included CD4+ T lymphocytes and CD8+ T lymphocytes in responses to either form of JE viral antigens. Responses to EPs were observed only with PBMC from some American vaccinees whose PBMC also responded to the virus and lysate. These results indicate that JE virus infection and immunization with an inactivated JE vaccine induce JE virus-specific CD4+ and CD8+ T memory lymphocytes that can be induced to proliferate by infectious JE virus and noninfectious JE antigens.

L31 ANSWER 12 OF 17 MEDLINE on STN

94367626. PubMed ID: 8085382. Avipox virus-vectored Japanese encephalitis virus vaccines: use as vaccine candidates in combination with purified subunit immunogens. **Konishi E**; Pincus S; Paoletti E; Shope R E; Wason P W. (Department of Medical Zoology, Kobe University School of Medicine, Japan.) Vaccine, (1994 May) 12 (7) 633-8. Journal code: 8406899. ISSN: 0264-410X. Pub. country: ENGLAND: United Kingdom. Language: English.

AB An avipox virus, canarypox (ALVAC), which is naturally host-range restricted, was used to construct recombinants encoding the Japanese encephalitis virus (JEV) **prM**, **E** and NS1 genes (vCP107) and **prM** and **E** genes (vCP140). Mice immunized with these recombinant viruses produced JEV neutralizing antibodies and were protected from lethal JEV challenge. Protection was also observed in mice immunized with a subunit vaccine candidate, consisting of extracellular particles (EPs; RNA-free subviral membrane vesicles containing **prM**/M and **E** proteins) derived from HeLa cell cultures infected with a JEV-vaccinia recombinant. Mice primed with vCP107 and boosted with EPs had higher antibody levels than mice immunized twice with EPs alone, although the levels were comparable to that obtained in mice immunized twice with the recombinant virus. Mice immunized with a mixture of recombinant virus (vCP107) plus EPs had neutralizing antibody titres higher than mice immunized with the recombinant virus or EPs alone.

L31 ANSWER 13 OF 17 MEDLINE on STN

93172590. PubMed ID: 8437257. Proper maturation of the Japanese encephalitis virus **envelope** glycoprotein requires cosynthesis with the **premembrane** protein. **Konishi E**; Mason P W. (Department of Medical Zoology, Kobe University School of Medicine, Japan.) Journal of virology, (1993 Mar) 67 (3) 1672-5. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB The role of the Japanese encephalitis virus (JEV) **premembrane** (**prM**) protein in maturation of the **envelope** (**E**) glycoprotein was evaluated by using recombinant vaccinia viruses encoding **E** in the presence (vP829) or absence (vP658) of **prM**. Immunofluorescence analyses showed that **E** appeared to be localized in the endoplasmic reticulum of cells infected with JEV, vP829, or vP658. However, reactivity with monoclonal antibodies and behavior in Triton X-114 indicated that **E** produced in the absence of **prM** behaved abnormally. Furthermore, **E** produced in the presence of **prM** by recombinant vaccinia viruses could be incorporated into flavivirus pseudotypes, whereas **E** synthesized in the absence of **prM** could not. These results demonstrate that cosynthesis of **prM** is required for proper folding, membrane association, and assembly of the flavivirus **E** protein.

L31 ANSWER 14 OF 17 MEDLINE on STN

92410626. PubMed ID: 1326813. A highly attenuated host range-restricted vaccinia virus strain, NYVAC, encoding the **prM**, **E**, and NS1 genes of Japanese encephalitis virus prevents JEV viremia in swine. **Konishi E**; Pincus S; Paoletti E; Laegreid W W; Shope R E; Mason P W. (Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, Connecticut 06510.) Virology, (1992 Sep) 190 (1) 454-8. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.

AB A highly attenuated strain of vaccinia virus (NYVAC) was engineered to express the Japanese encephalitis virus (JEV) **prM**, **E**, and NS1 genes or the **prM** and **E** genes. The recombinant viruses were tested as vaccine candidates in pigs, a natural host of JEV. JEV-neutralizing and hemagglutination-inhibiting antibodies appeared in swine sera 7 days after immunization with 10(8) PFU of the recombinant viruses and increased after a second dose at 28 days. The JEV levels detected in the serum after JEV challenge (d56) of the swine with 2 x 10(5) PFU of JEV were significantly reduced in animals inoculated with the recombinant viruses. These results demonstrate the ability of these NYVAC-vectored recombinants to protect pigs from JEV viremia.

L31 ANSWER 15 OF 17 MEDLINE on STN

92263775. PubMed ID: 1585642. Mice immunized with a subviral particle containing the Japanese encephalitis virus **prM**/M and **E** proteins are protected from lethal JEV infection. **Konishi E**; Pincus S; Paoletti E; Shope R E; Burrage T; Mason P W. (Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, Connecticut 06510.) Virology, (1992 Jun) 188 (2) 714-20. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.

AB Extracellular subviral particles produced by HeLa cells infected with a recombinant vaccinia virus encoding the **prM** and **E** genes of Japanese encephalitis virus (JEV) were purified and characterized. These particles contained the JEV **prM**/M and **E** proteins embedded in a lipid bilayer, and RNA was not detected in particles using the polymerase chain reaction and primers recognizing a part of the JEV **E** gene. The particles were uniformly spherical with a 20-nm diameter and had 5-nm projections on their surface. Mice that received a single inoculation of the purified extracellular particles emulsified with Freund's complete adjuvant were fully protected against 4.9 x 10(5) LD50 of JEV. Comparison of the neutralizing and hemagglutination-inhibiting antibody titers and radioimmunoprecipitation data showed that immunization with the particles induced an immune response similar to that following inoculation with the recombinant vaccinia virus.

L31 ANSWER 16 OF 17 MEDLINE on STN

92142515. PubMed ID: 1736531. Recombinant vaccinia virus producing the

prM and **E** proteins of yellow fever virus protects mice from lethal yellow fever encephalitis. Pincus S; Mason P W; **Konishi E**; Fonseca B A; Shope R E; Rice C M; Paoletti E. (Virogenetics Corporation, Troy, New York 12180.) Virology, (1992 Mar) 187 (1) 290-7. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.

AB Four recombinant vaccinia viruses were constructed for expression of different portions of the 17D yellow fever virus (YFV-17D) open reading frame. A recombinant, vP869, expressing **prM** and **E** induced high titers of neutralizing and hemagglutination inhibiting antibodies in mice and was protective against intracranial challenge with the French neurotropic strain of YFV. Levels of protection were equivalent to those achieved by immunization with the YFV-17D vaccine virus. Recombinant vaccinia viruses expressing **E** and NS1, C **prM**, **E**, NS1, or only NS1 failed to protect mice against challenge with YFV despite eliciting antibodies to NS1. The vP869-infected HeLa cells produced a particulate extracellular hemagglutinin (HA) similar to that produced by YFV-infected cells, supporting previous studies with Japanese encephalitis virus (Mason et al., 1991), suggesting that the ability of recombinant vaccinia virus to produce extracellular HA particles is important for effective flavivirus immunity.

L31 ANSWER 17 OF 17 MEDLINE on STN

92024099. PubMed ID: 1833876. Comparison of protective immunity elicited by recombinant vaccinia viruses that synthesize **E** or NS1 of Japanese encephalitis virus. **Konishi E**; Pincus S; Fonseca B A; Shope R E; Paoletti E; Mason P W. (Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, Connecticut 06510.) Virology, (1991 Nov) 185 (1) 401-10. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.

AB Immunization with recombinant vaccinia viruses that specified the synthesis of Japanese encephalitis virus (JEV) glycoproteins protected mice from a lethal intraperitoneal challenge with JEV. Recombinants which coexpressed the genes for the structural glycoproteins, **prM** and **E**, elicited high levels of neutralizing (NEUT) and hemagglutination inhibiting (HAI) antibodies in mice and protected mice from a lethal challenge by JEV. Recombinants expressing only the gene for the nonstructural glycoprotein, NS1, induced antibodies to NS1 but provided low levels of protection from a similar challenge dose of JEV. Antibodies to the NS3 protein in postchallenge sera, representing the degree of infection with challenge virus, were inversely correlated to NEUT and HAI titers and levels of protection. These results indicate that although vaccinia recombinants expressing NS1 can provide some protection from lethal JEV infection, recombinants expressing **prM** and **E** elicited higher levels of protective immunity.

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FILE 'USPATFULL' ENTERED AT 15:59:07 ON 30 APR 2004
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E CHANG G J/IN
L2 106 S E3
L3 2 S L2 AND FLAVIVIR?

FILE 'MEDLINE' ENTERED AT 16:01:13 ON 30 APR 2004
E CHANG G J/AU
L4 49 S E3
L5 29 S L4 AND (FLAVIVIR? OR YELLOW OR DENGUE OR ENCEPHALITIS)
L6 9 S L5 AND (PRM OR E OR PRM/M OR PRM/E)

FILE 'USPATFULL' ENTERED AT 16:06:37 ON 30 APR 2004

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L7          3470 S (FLAVIVIR? OR YELLOW FEVER VIR? OR DENGUE OR ENCEPHALITIS VIR
L8            79 S L7 AND (PRM OR PREMEMBRANE)
L9            79 S L8 AND (E OR ENVELOPE)
L10           79 S L9 AND (M OR MEMBRANE)
L11           43 S L10 AND (SIGNAL SEQUENCE)
L12            5 S L11 AND KOZAK
L13           38 S L11 NOT L12
L14           15 S L13 AND AY<1999
L15          805 S L7 AND (KOZAK OR RIBOSOME BINDING SITE)
L16            2 S L15 AND KOZAK/CLM
L17            0 S L15 AND (KOZAK RIBOSOME BINDING SITE? OR KOZAK CONCENSUS SEQU
L18          716 S L15 AND (CMV OR CYTOMEGALOVIRUS)
L19          191 S L18 AND (IE OR IMMEDIATE-EARLY PROMOTER?)
L20           30 SS L19 AND (CMV/CLM OR CYTOMEGALOVIRUS/CLM)
L21            2 S L20 AND (IE/CLM OR IMMEDIATE-EARLY/CLM)
L22           28 S L20 NOT L21
L23           10 S L20 AND AY<1999
L24          743 S L15 AND (POLYADENYLATION OR POLY-A)
L25          423 S L24 AND (POLYADENYLATION (5W) TERMINAT?)
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L27            1 S E4
                 E KOZAK M/IN
L28            1 S E5

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(FILE 'HOME' ENTERED AT 15:58:13 ON 30 APR 2004)

FILE 'USPATFULL' ENTERED AT 15:59:07 ON 30 APR 2004
E CHANG GWONG JEN/IN
L1 1 S E4

FILE 'WPIDS' ENTERED AT 16:00:09 ON 30 APR 2004
E CHANG G J/IN
L2 106 S E3
L3 2 S L2 AND FLAVIVIR?

FILE 'MEDLINE' ENTERED AT 16:01:13 ON 30 APR 2004
E CHANG G J/AU
L4 49 S E3
L5 29 S L4 AND (FLAVIVIR? OR YELLOW OR DENGUE OR ENCEPHALITIS)
L6 9 S L5 AND (PRM OR E OR PRM/M OR PRM/E)

FILE 'USPATFULL' ENTERED AT 16:06:37 ON 30 APR 2004
L7 3470 S (FLAVIVIR? OR YELLOW FEVER VIR? OR DENGUE OR ENCEPHALITIS VIR
L8 79 S L7 AND (PRM OR PREMEMBRANE)
L9 79 S L8 AND (E OR ENVELOPE)
L10 79 S L9 AND (M OR MEMBRANE)
L11 43 S L10 AND (SIGNAL SEQUENCE)
L12 5 S L11 AND KOZAK
L13 38 S L11 NOT L12
L14 15 S L13 AND AY<1999
L15 805 S L7 AND (KOZAK OR RIBOSOME BINDING SITE)
L16 2 S L15 AND KOZAK/CLM
L17 0 S L15 AND (KOZAK RIBOSOME BINDING SITE? OR KOZAK CONCENSUS SEQU
L18 716 S L15 AND (CMV OR CYTOMEGALOVIRUS)
L19 191 S L18 AND (IE OR IMMEDIATE-EARLY PROMOTER?)
L20 30 SS L19 AND (CMV/CLM OR CYTOMEGALOVIRUS/CLM)
L21 2 S L20 AND (IE/CLM OR IMMEDIATE-EARLY/CLM)
L22 28 S L20 NOT L21
L23 10 S L20 AND AY<1999
L24 743 S L15 AND (POLYADENYLATION OR POLY-A)
L25 423 S L24 AND (POLYADENYLATION (5W) TERMINAT?)
L26 2 S L25 AND L23
E KONISHI E/IN
L27 1 S E4
E KOZAK M/IN
L28 1 S E5

FILE 'MEDLINE' ENTERED AT 16:45:49 ON 30 APR 2004
E KONISHI E/AU
L29 102 S E3-E5
L30 17 S L29 AND (PRM OR PREMEMBRANE)
L31 17 S L30 AND (E OR ENVELOPE OR ENV)

FILE 'USPATFULL' ENTERED AT 16:53:49 ON 30 APR 2004

=> s l15 and (DNA vaccin? or genetic immunizat? or DNA immuniz?)
104058 DNA
36087 VACCIN?
1768 DNA VACCIN?
(DNA(W)VACCIN?)
75185 GENETIC
25874 IMMUNIZAT?
961 GENETIC IMMUNIZAT?

(GENETIC(W)IMMUNIZAT?)
104058 DNA
36036 IMMUNIZ?
1146 DNA IMMUNIZ?
(DNA(W)IMMUNIZ?)
L32 460 L15 AND (DNA VACCIN? OR GENETIC IMMUNIZAT? OR DNA IMMUNIZ?)

=> s l32 and l23
L33 6 L32 AND L23

=> d l33,cbib,ab,kwic

L33 ANSWER 1 OF 6 USPATFULL on STN
2001:107871 **DNA vaccines** against tick-borne **flaviviruses**.
Schmaljohn, Connie S., Frederick, MD, United States
The United States of America as represented by the Secretary of the Army, Washington, DC, United States (U.S. corporation)
US 6258788 B1 20010710
APPLICATION: US 1998-197218 19981120 (9) <--
PRIORITY: US 1997-65750P 19971120 (60)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Particle mediated immunization of tick-borne **flavivirus** genes confers homologous and heterologous protection against tick borne encephalitis.
TI **DNA vaccines** against tick-borne **flaviviruses**
AI US 1998-197218 19981120 (9) <--
AB Particle mediated immunization of tick-borne **flavivirus** genes confers homologous and heterologous protection against tick borne encephalitis.
SUMM . . . over a wide area of Europe and the former Soviet Union. TBE is most frequently caused by infection with the **flaviviruses** Central European encephalitis (CEE) virus, or Russian spring summer encephalitis (RSSE) virus. These viruses are antigenically and genetically closely related. . .
SUMM . . . candidate vaccines, which express the premembrane (prM) and envelope (E) genes of RSSE or CEE viruses under control of a **cytomegalovirus** early promoter. We chose the prM and E genes for expression because of earlier reports with other **flaviviruses** which indicated that coexpressed prM and E form subviral particles that are able to elicit neutralizing and protective immune responses. . .
SUMM To deliver our **DNA vaccines**, we chose to use the PowderJect-XR.TM. gene gun device described in WO 95/19799, Jul. 17, 1995. This instrument, which delivers. . . Results Cancer Res. 128:45). In this application we describe the elicitation of cross-protective immunity to RSSE and CEE viruses by **DNA vaccines**.
SUMM . . . eliciting in an individual an immune response against an alphavirus which causes tick-borne encephalitis comprising delivering to the individual a **DNA vaccine** comprising a vector including a viral antigen such that when the antigen is introduced into a cell from the individual,. . .
SUMM In one aspect of the invention, the **DNA vaccine** is delivered by coating a small carrier particle with the **DNA vaccine** and delivering the DNA-coated particle into an animal's epidermal tissue via particle bombardment. This method may be adapted for delivery. . .
SUMM The **DNA vaccine** according to the present invention is inherently safe, is not painful to administer, and should not result in adverse side effects to the vaccinated individual. In addition, the invention does not require growth or use of tick-borne **flavivirus**, which may be spread by aerosol transmission and are typically fatal.
DRWD . . . are similar to those of pWRG1602 described previously (Dimmock, N. J., 1995, Med. Virol. 5: 165) and include a human **cytomegalovirus** early promoter (**CMV IE** promoter) and intron A, a bovine growth hormone transcription terminator and polyadenylation signal (BGH pA), and a kanamycin resistance gene.
DRWD FIGS. 3A and B. Antibody responses of mice to naked **DNA vaccines** as detected by ELISA.
DRWD FIGS. 5A, B and C. Plaque reduction neutralization by pre- and

postchallenge sera of mice immunized with naked **DNA vaccines** expressing the prM and E genes of RSSE (FIG. 5A), CEE (FIG. 5B) or RSSE and CEE (FIG. 5C) viruses.. . .

DRWD . . . precipitation of radiolabeled Langat virus proteins with pre- (lanes 1) and postchallenge (lanes 2) sera from mice vaccinated with naked **DNA vaccines** expressing the prM and E genes of CEE, RSSE or RSSE and CEE viruses. Immune precipitation products were analyzed by. . .

DETD **DNA vaccination** mimicks the de novo antigen production and MHC class I-restricted antigen presentation obtainable with live vaccines, without the risks of pathogenic infection. **DNA vaccination** involves administering antigen-encoding polynucleotides in vivo to induce the production of a correctly folded antigen(s) within the target cells. The introduction of the **DNA vaccine** will cause to be expressed within those cells the structural protein determinants associated with the pathogen protein or proteins. The. . .

DETD . . . subunit vaccines which do not elicit a cytotoxic response necessary to prevent the establishment of infection or disease. Also, this **DNA vaccine** approach allows delivery to mucosal tissues which may aid in conferring resistance to viral introduction since entry of the virus. . .

DETD In order to achieve the immune response sought, a **DNA vaccine** construct capable of causing transfected cells of the vaccinated individual to express one or more major viral antigenic determinant is. . .

DETD In one embodiment, the present invention relates to a DNA or cDNA segment which encodes an antigen from a tick-borne **flavivirus** such as RSSE, CEE, or Langat. More specifically, prM and E genes of CEE were deduced from the CEE viral. . .

DETD . . . were modified around the translation initiation codon (bold type below) to generate sequences with a favorable context for translation initiation (**Kozak**, M., 1989, J. Cell. Biol. 108:229). The forward and reverse primers for RSSE were: 5'GCAGTAGACAGGATGGGTTGGTTG3' (SEQ ID NO:3) and 5'GCACAGCCAACTTAAGCTCCCACTCC3'. . .

DETD . . . affecting the ability of the construct to achieve the desired effect, namely induction of a protective immune response against tick-borne **flavivirus** challenge. It is further understood in the art that certain advantageous steps can be taken to increase the antigenicity of. . . by modifying the genetic sequence encoding the protein. It is contemplated that all such modifications and variations of the tick-borne **flavivirus** glycoprotein genes are equivalents within the scope of the present invention.

DETD . . . (Konishi, E. et al., 1992, Virology 188:714), or any expression vector such as viral vectors e.g. adenovirus or Venezuelan equine **encephalitis virus** and others known in the art. Preferably, a promoter sequence operable in the target cells is operably linked to the. . . 5', or upstream, of the coding sequence for the encoded protein to be expressed. A suitable promoter is the human **cytomegalovirus immediate early promoter**. A downstream transcriptional terminator, or polyadenylation sequence, such as the polyA addition sequence of the bovine growth hormone gene, may. . .

DETD . . . the method of the present invention is pWRG7077 (4326 bp)(PowderJect Vaccines, Inc., Madison, Wisc.), FIG. 1. pWRG7077 includes a human **cytomegalovirus** (hCMV) **immediate early promoter** and a bovine growth hormone polyA addition site. Between the promoter and the polyA addition site is Intron A, a sequence that naturally occurs in conjunction with the hCMV **IE** promoter that has been demonstrated to increase transcription when present on an expression plasmid. Downstream from Intron A, and between. . .

DETD In the present invention, the **DNA vaccine** is transferred into the susceptible individual by means of an accelerated particle gene transfer system. The technique of accelerated particles. . .

DETD . . . impact or effect on the treated individual. Therefore, the accelerated particle method is also preferred in that it allows a **DNA vaccine** capable of eliciting an immune response to be directed both to a particular tissue, and to a particular cell layer. . .

carboxy terminal 57% **E**, generating YF99 containing 21 amino acids of C, **prM**, **E** in the HA locus. Donor plasmid YF53 was transfected into vP913 (NYVAC-MV) infected cells to generate the vaccinia recombinant vP997.

DETD Cloning of **Dengue** Type 1 Into a Vaccinia Virus Donor Plasmid. Plasmid DEN1 containing DEN cDNA encoding the carboxy-terminal 84% NS1 and amino-terminal. . . digested pUC8. An EcoRI-HindIII fragment from DEN1 (nucleotides 2559-3745) and SacI-EcoRI fragment of DEN cDNA encoding the carboxy-terminal 36% of **E** and amino-terminal 16% NS1 (nucleotides 1447-2559, Mason et al., 1987b) were ligated to HindIII -SacI digested IBI24 (International Biotechnologies, Inc., New Haven, Conn.) generating DEN3 encoding the carboxy-terminal 64% **E** through amino-terminal 45% NS2A with a base missing in NS1 (nucleotide 2467).

DETD . . . an AvaI-SacI fragment of DEN cDNA (nucleotides 424-1447 Mason et al., 1987) generating DEN4 encoding the carboxy-terminal 11 aa C, **prM** and amino-terminal 36% **E**.

DETD Plasmid DEN6 containing DEN cDNA encoding the carboxy-terminal 64% **E** and amino-terminal 18% NS1 (nucleotides 1447-2579 with nucleotide 2467 present Mason et al., 1987b) was derived by cloning a SacI-XhoI. . . Biotechnologies, Inc., New Haven, Conn.). Plasmid DEN15 containing DEN cDNA encoding 51 bases of the DEN 5' untranslated region, C, **prM** and amino-terminal 36% **E** was derived by cloning a HindIII-SacI fragment of DEN cDNA (nucleotides 20-1447, Mason et al., 1987b) into HindIII-SacI digested IBI25.. . .

DETD . . . change the following potential vaccinia virus early transcription termination signals (Yuen et al., 1987). The two T5NT sequences in the **prM** gene in DEN4 were mutagenized (1) 29 aa from the carboxy-terminus (nucleotides 822-828 TTTTTCT to TATTTCT) and (2) 13 aa. . .

DETD . . . 4102) in plasmid DEN23 creating DEN24, (2) to insert a SmaI site and ATG 15 aa from the carboxy-terminus of **E** in DEN7 (nucleotide 2348) creating DEN10, (3) to insert an EagI and HindIII site at the carboxy-terminus of NS2B (nucleotide. . .

DETD . . . DEN7 (nucleotides 1447-2579) was substituted for the corresponding region in DEN3 generating DEN19 containing DEN cDNA encoding the carboxy-terminal 64% **E** and amino-terminal 45% NS2A (nucleotides 1447-3745) with nucleotide 2467 present and the modified transcription termination signal (nucleotides 2448-2454). A XhoI-XbaI. . .

DETD A HindIII-PstI fragment of DEN16 (nucleotides 20-494) was ligated to a HindIII-PstI fragment from DEN47 (encoding the carboxy-terminal 83% **prM** and amino-terminal 36% of **E** nucleotides 494-1447 and plasmid origin of replication) generating DEN17 encoding C, **prM** and amino-terminal 36% **E** with part of the H6 promoter and EcoRV site preceding the amino-terminus of C. A HindIII-BglII fragment from DEN17 encoding the carboxy-terminal 13 aa C, **prM** and amino-terminal 36% **E** (nucleotides 370-1447) was ligated to annealed oligonucleotides SP111 and SP112 (containing a disabled HindIII sticky end, EcoRV site to -1. . . a BglII sticky end) creating DEN33 encoding the EcoRV site to -1 of the H6 promoter, carboxy-terminal 20 aa C, **prM** and amino-terminal 36% **E**.

DETD . . . digested pTP15 (Mason et al., 1991) was ligated to a SmaI-SacI fragment from DEN4 encoding the carboxy-terminal 11 aa C, **prM** and amino-terminal 36% **E** (nucleotides 377-1447) and SacI-EagI fragment from DEN3 encoding the carboxy-terminal 64% **E**, NS1 and amino-terminal 45% NS2A generating DENL. The SacI-XhoI fragment from DEN7 encoding the carboxy-terminal 64% **E** and amino-terminal 18% NS1 (nucleotides 1447-2579) was ligated to a BstEII-SacI fragment from DEN47 (encoding the carboxy-terminal 55% **prM** and amino-terminal 36% **E** nucleotides 631-1447) and a BstEII-XhoI fragment from DENL (containing the carboxy-terminal 11 aa C, amino-terminal 45% **prM**, carboxy-terminal 82% NS1, carboxy-terminal 45% NS2A, origin of replication and vaccinia sequences) generating DENS. A unique SmaI site (located between. . .

DETD . . . an EcoRV-SacI fragment of DEN8VC (containing vaccinia sequences, H6 promoter from -21 to -124, origin of replication and amino-terminal 64% **E**, NS1, amino-terminal 45% NS2A nucleotides

1447-3745) generating DEN10. A XhoI-EagI fragment from DEN25 (nucleotides 2579-4102) was ligated to an XhoI-EagI fragment of DEN18 (containing the origin of replication, vaccinia sequences and DEN C, **prM**, **E** and amino-terminal 18% NS1 nucleotides 68-2579) generating DEN26. An EcoRV-SacI fragment from DEN8VC (positions -21 to -1 H6 promoter DEN. . . to an EcoRV-SacI fragment of DEN26 (containing the origin of replication, vaccinia sequences and DEN region encoding the carboxy-terminal 64% **E**, NS1 and NS2A with a base missing in NS1 at nucleotide 2894) generating DEN32. DEN32 was transfected into vP410 infected. . .

DETD . . . DEN10 (nucleotides 1447-2579) was substituted for the corresponding region in DEN3 generating DEN11 containing DEN cDNA encoding the carboxy-terminal 64% **E**, NS1 and amino-terminal 45% NS2A with a SmaI site and ATG 15 aa from the carboxy-terminus of **E**. A SmaI-EagI fragment from DEN11 (encoding the carboxy-terminal 15 aa **E**, NS1 and amino-terminal 45% NS2A nucleotides 2348-3745) was ligated to SmaI-EagI digested pTP15 generating DEN12.

DETD An EcoRV-XhoI fragment from DEN8VC (positions -21 to -1 H6 promoter DEN nucleotides 377-2579 encoding the carboxy-terminal 11 aa C, **prM E**, amino-terminal 18% NS1) was ligated to an EcoRV-XhoI fragment from DEN31 (containing the origin of replication, vaccinia sequences and DEN. . . An EcoRV-SacI fragment from DEN33 (positions -21 to -1 H6 promoter DEN nucleotides 350-1447 encoding the carboxy-terminal 20 aa C, **prM** and amino-terminal 36% **E**) and a SacI-XhoI fragment from DEN32 (encoding the carboxy-terminal 64% **E** and amino-terminal 18% NS1 nucleotides 1447-2579) were ligated to the EcoRV-SacI fragment from DEN31 described above generating DEN 34. DEN34. . .

DETD . . . the left terminus of vaccinia and by introducing a deletion near the right terminus. All deletions were accomplished using the **E**. coli guanine phosphoribosyl transferase gene and mycophenolic acid in a transient selection system.

DETD For use as a selectable marker, the **E**. coli gene encoding guanine phosphoribosyl transferase (Ecogpt) (Pratt et al., 1983) was placed under the control of a poxvirus promoter.. . .

DETD . . . subunit of ribonucleotide reductase (Slabaugh et al., 1988). Also included in this deletion is ORF F2L, which shows homology to **E**. coli dUTPase, another enzyme involved in nucleotide metabolism (Goebel et al., 1990a,b). F2L also shows homology to retroviral protease (Slabaugh. . .

DETD . . . sequences, the predicted translation product of Copenhagen ORF B16 is truncated at the amino terminus and does not contain a **signal sequence**. B19R encodes a vaccinia surface protein (S antigen) expressed at early times post infection (Ueda et al., 1990). Both B16R. . .

DETD . . . immunological assays was comprised of RPMI 1640 medium supplemented with 10% FBS, 4 mM L-glutamine, 20 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonate), 5×10^{-5} **M** 2-mercaptoethanol, 100 IU penicillin per ml, and 100 μg/ml streptomycin. Stim Medium was comprised of Eagle's Minimum Essential Medium supplemented with 10% FBS, 4 mM L-glutamine, 10^{-4} **M** 2-mercaptoethanol, 100 IU penicillin per ml, and 100 μg streptomycin per ml.

DETD ALVAC and NYVAC Recombinants Containing the V3 Loop and Epitope 88 of the HIV-1 (IIIB) **Envelope**. A 150 bp fragment encompassing the V3 loop (amino acids 299-344; Javeherian et al., 1989) of HIV-1 (IIIB) was derived. . .

DETD . . . isolated by phenol extraction (2×) and ether extraction (1×). The isolated fragment was blunt-ended using the Klenow fragment of the **E**. coli DNA polymerase in the presence of 2 mM dNTPs. The fragment was ligated to pSD550, a derivative of pSD548. . .

DETD ALVAC- and NYVAC-Based Recombinants Expressing the HIV-1 (IIIB) **Envelope** Glycoproteins. An expression cassette composed of the HIV-1 (IIIB) u gene juxtaposed 3' to the vaccinia virus H6 promoter (Guo. . .

DETD . . . pBSHIV3BEAII was digested with NruI and XbaI. The derived 2.7 kb fragment was blunt-ended with the Klenow fragment of the **E**. coli

DNA polymerase in the presence of 2 mM dNTPs. This fragment contains the entire HIV-1 env gene juxtaposed 3'. . .

DETD . . . followed by a partial KpnI digestion. The 1.6 kb fragment was blunt-ended by treatment with the Klenow fragment of the **E**. coli DNA polymerase in the presence of 2 mM dNTPs. This fragment was inserted into pSD54IVC digested with SmaI to. . .

DETD . . . Vero cells monolayers were either mock infected, infected with the parental virus vP866, or infected with recombinant virus at an **m**.o.i. of 10 PFU/cell. Following a 1 hr adsorption period, the inoculum was aspirated and the cells were overlayed with 2. . .

DETD . . . using sera pooled from HIV-1 seropositive individuals showed specific precipitation of the gp120 and gp41 mature forms of the gp160 **envelope** glycoprotein from vP911 infected cell lysates. No such specific gene products were detected in the parentally (NYVAC; vP866) infected cell. . .

DETD . . . for 1 hour in tissue culture medium containing 2% FBS at 37° C. with the appropriate vaccinia virus at a **m**.o.i. of 25 pfu per cell. Following infection, the stimulator cells were washed several times in Stim Medium and diluted to. . .

DETD . . . cells were infected overnight by incubation at 1×10^7 cells per ml in tissue culture medium containing 2% FBS at a **m**.o.i. of 25 pfu per cell for 1 hour at 37° C. Following incubation, the cells were diluted to between $1\text{-}2\times10^6$. . .

DETD

	. . . ± 7.1*	-4.0	
	1.8	2.2	1.2
vP911	-4.0 ± 4.6*	1.4	
	2.5	2.0	5.1
vP921	-3.4 ± 10.7*	15.5*	
	0.9	1.5	2.8

E:T = 100:1

*P < 0.05 vs appropriate controls, Student's ttest

DETD . . . plasmid vector, pIBI25 (International Biotechnologies, Inc., New Haven, Conn.), generating plasmid pIBI25env. Recombinant plasmid pIBI25env was used to transform competent **E**. coli CJ236 (dut- ung-) cells. Single-stranded DNA was isolated from phage derived by infection of the transformed **E**. coli CJ236 cells with the helper phage, MG408. This single-stranded template was used in vitro mutagenesis reactions (Kunkel et al.,. . .

DETD . . . 2.5 kb (envIS+) and 2.4 kb (envIS-), respectively, were isolated and blunt-ended by reaction with the Klenow fragment of the **E**. coli DNA polymerase in the presence of 2 mMdNTPs. These fragments were ligated with the 3.5 kb fragment derived by digestion of pSIVenvVV with NruI and PstI with a subsequent blunting step with the Klenow fragment of the **E**. coli DNA polymerase in the presence of 2 mM dNTPs. The plasmid pSIVenvVV contains the SIV env gene expression cassette. . .

DETD . . . seropositive individuals were performed as described in Materials and Methods. All six recombinants directed the synthesis of the HIV-1 gp161 **envelope** precursor. The efficiency of processing of gp160 to gp120 and gp41, however, varied between cell types and was also affected. . .

DETD . . . to yield pBSH6HIV2ENV. The 2.7 kb HindIII/XbaI insert from pBSH6HIV2ENV was isolated and blunt-ended with the Klenow fragment of the **E**. coli DNA polymerase in the presence of 2 mM dNTP. The blunt-ended fragment was inserted into a SmaI digested pSD5HIVC. . .

DETD . . . gp160. Vero cell monolayers were either mock infected, infected with the parental virus vP866, or infected with vP920 at an **m**.o.i. of 10 PFU/cell. Following an hour adsorption period, the inoculum was aspirated and the cells were overlayed with 2 mls. . .

DETD Human sera from HIV-2 seropositive individuals specifically precipitated the HIV-2 gp160 **envelope** glycoprotein from vP920 infected cells. Furthermore, the authenticity of the expressed HIV-2 env gene product was confirmed, since the gp160. . .

DETD . . . coding sequence juxtaposed 3' to the vaccinia virus H6 promoter. This fragment was blunted with the Klenow fragment of the **E**.

coli DNA polymerase in the presence of 2 mM dNTPs. The blunt ended fragment was ligated to SmaI digested pSDSHIVC to. . .

DETD . . . digestion with HindIII liberated a 2.7 kb HindIII/EcoRI fragment. This fragment was blunt-ended by treatment with Klenow fragment of the **E**. coli DNA polymerase in the presence of 2 mM dNTPs. The fragment was ligated into pSD550VC digested with SmaI. The. . .

DETD Expression Analyses. The SIV gp140 env gene product is a typical glycoprotein associated with the plasma **membrane** of infected cells. It is expressed as a polyprotein of 140 kDa that is proteolytically cleaved to an extracellular species. . .

DETD . . . and gag) in Vero cells infected with the NYVAC/HIV recombinants was analyzed by immunoprecipitation. Vero cells were infected at an **m**.o.i. of 10 with the individual recombinant viruses, with the NYVAC parent virus, or were mock infected. After a 1 hour. . .

DETD The plasmid pF7D3 was linearized with XhoI and blunt-ended with the Klenow fragment of the **E**. coli DNA polymerase in the presence of 2 mM dNTPs. This linearized plasmid was ligated with annealed oligonucleotides F7MCSB (SEQ. . .

DETD . . . the H6 promoter) and PstI. The 3.5 kb resultant fragment was isolated and blunt-ended using the Klenow fragment of the **E**. coli DNA polymerase in the presence of 2 mM dNTPs. This blunt-ended fragment was ligated to a 1700 bp EcoRV/HpaI. . .

DETD . . . HA molecule is synthesized and glycosylated as a precursor molecule at the rough endoplasmic reticulum. During passage to the plasma **membrane** it undergoes extensive post-translational modification culminating in proteolytic cleavage into the disulphide linked HA_1 and HA_2 subunits and insertion into the host cell **membrane** where it is subsequently incorporated into mature viral envelopes. To determine whether the HA molecules produced in cells infected with. .

DETD . . . 3'end-EcoRV fragment (D). Plasmid pVHAH6g13 was digested with BglII and KpnI to isolate the 1330 bp BglII-H6-EHV-1 gC 5'-KpnI fragment (**E**).

DETD Fragments C, D and **E** were finally ligated together into vector pSD541VC digested with BglII and XhoI to produce plasmid pJCA042. Plasmid pJCA042 is the. . .

DETD . . . region-BamHI fragment (L). Plasmid pVHAH6g13 was digested with BglII and XhoI to isolate the 440 bp BglII-H6-EHV-1 gC 5'portion-XhoI fragment (**M**). Fragments K, L and **M** were then ligated together to produce plasmid pJCA040.

DETD . . . authentic BHV1 gIV glycoprotein. Vero cell monolayers were either mock infected, infected with NYVAC or infected with vP1051 at an **m**.o.i. of 10 PFU/cell. Following an hour adsorption period, the inoculum was aspirated and the cells were overlayed with 2 mls. . .

DETD . . . gene was then cloned into pIBR8. This was accomplished by cloning the 2,285 bp StuI fragment of pIBRS6 into the **E**. coli DNA polymerase I (Klenow fragment) filled-in 4,300 bp StuI-BglII (partial) fragment of pIBR8. The plasmid generated by this manipulation. . .

DETD The H6-promoted BHV1 gI gene was then moved to a vaccinia virus donor plasmid. This was accomplished by cloning the **E**. coli DNA polymerase I (Klenow fragment) filled-in 2,900 bp BglII-NCoI (partial) fragment of pIBR20 into the SmaI site of pSD542.. . .

DETD . . . gI and gIV glycoproteins. Vero cell monolayers were either mock infected, infected with NYVAC or infected with vP1074 at an **m**.o.i. of 10 PFU/cell. Following an hour adsorption period, the inoculum was aspirated and the cells were overlayed with 2 mls. . .

DETD . . . authentic BHV1 gIII glycoprotein. Vero cell monolayers were either mock infected, infected with NYVAC or infected with vP1073 at an **m**.o.i. of 10 PFU/cell. Following an hour adsorption period, the inoculum was aspirated and the cells were overlayed with 2 mls. . .

DETD . . . gIII and gIV glycoproteins. Vero cell monolayers were either mock infected, infected with NYVAC or infected with vP1083 at an **m**.o.i. of 10 PFU/cell. Following an hour adsorption period, the inoculum was aspirated and the cells were overlayed with 2 mls. . .

DETD . . . gI and gIII glycoproteins. Vero cell monolayers were either mock infected, infected with NYVAC or infected with vP1087 at an

m.o.i. of 10 PFU/cell. Following an hour adsorption period, the inoculum was aspirated and the cells were overlayed with 2 mls. . .

DETD . . . gIII and gIV glycoproteins. Vero cell monolayers were either mock infected, infected with NYVAC or infected with vP1079 at an **m**.o.i. of 10 PFU/cell. Following an hour adsorption period, the inoculum was aspirated and the cells were overlayed with 2 mls. . .

DETD . . . the 1,370 bp EcoRI-BamHI fragment of pSP65-gE1 (obtained from Eurogentec, Liege, Belgium; Renard et al., European Patent Application No:86870095) with **E**. coli DNA polymerase I (Klenow fragment), ligating XhoI linkers onto the ends and cloning the resulting fragment into the XhoI. . .

DETD . . . gel and gE2 glycoproteins. Vero cell monolayers were either mock infected, infected with NYVAC or infected with vP972 at an **m**.o.i. of 10 PFU/cell. Following an hour adsorption period, the inoculum was aspirated and the cells were overlayed with 2 mls. . .

DETD . . . cloned into pIBI25. This was accomplished by blunt-ending the 1,370 bp EcoRI-BamHI fragment of pSP65-gE1, containing the gel "gene", with **E**. coli DNA polymerase I (Klenow fragment), ligating XhoI linkers onto the ends and cloning the resulting fragment into the XhoI. . .

DETD . . . cloned into pIBI25. This was accomplished by blunt-ending the 1,370 bp EcoRI-BamHI fragment of pSP65-gE1, containing the gel "gene", with **E**. coli DNA polymerase I (Klenow fragment), ligating XhoI linkers onto the ends and cloning the resulting fragment into the XhoI. . .

DETD . . . pig polyclonal serum followed by fluorescein isothiocyanate goat anti-guinea pig. Cells infected with vP1001 showed gB expressed on the plasma **membrane**. Weak internal expression was detected within cells infected with vCP139.

DETD . . . gene was excised from pED3 with NruI and XhoI and the purified fragment was cloned into pVQH6CP3L (plasmid described in **Flavivirus** section) cut with NruI and XhoI. The resulting plasmid, pC3-VP2, contains the H6 promoted VP2 gene flanked by the C3. . .

DETD . . . with EcoRI, which recognizes a unique EcoRI site within the canarypox sequences, and blunt-ended using the Klenow fragment of the **E**. coli DNA polymerase. The resultant plasmid was designated as pCPCV1. This plasmid contains the vaccinia virus H6 promoter followed by. . .

DETD An M13 clone containing the hemagglutinin (HA) gene from equine influenza virus (A2/Suffolk/89) was provided by Dr. **M**. Binns (Animal Health Trust, P.O. Box 5, Newmarket, Suffolk, CB8 7DW, United Kingdom). This clone contains a full-length 1.7 kb. . .

DETD . . . recombination tests with vP425 as the rescuing virus to construct a recombinant vaccinia virus (vP453) which expresses the entire FeLV **envelope** glycoprotein.

DETD . . . tests with vP410 as the rescuing virus to generate vP456. This vaccinia virus recombinant was generated to express the entire **envelope** glycoprotein lacking the putative immunosuppressive region.

DETD . . . of the H6 promoter sequence. The PstI site is located 420 bp downstream from the translation termination signal for the **envelope** glycoprotein open reading frame.

DETD . . . of the H6 promoter sequence. The HpaI site is located 180 bp downstream from the translation termination signal for the **envelope** glycoprotein open reading frame. These isolated fragments were blunt-ended. These 2.2 kbp H6/FeLV env sequences were inserted into the nonessential. . .

DETD . . . with EcoRI, which recognizes a unique EcoRI site within the canarypox sequences, and blunt-ended using the Klenow fragment of the **E**. coli DNA polymerase. The resultant plasmid was designated as pCPCV1. This plasmid contains the vaccinia virus H6 promoter followed by. . .

DETD The putative immunosuppressive region is situated within the p15E transmembrane region of the FeLV **envelope** glycoprotein (Cianciolo et al., 1986; Mathes et al., 1978). This region was deleted in the following manner. The FeLV-A env. . .

DETD . . . into the SmaI site of pSD553. This insertion was performed following blunt-ending the fragment with the Klenow fragment of the **E**. coli DNA polymerase in the presence of 2 mM dNTPs.

DETD immunoprecipitation. Vero cell monolayers were infected at an m.o.i. equal to 10 pfu/cell with parental or recombinant viruses. At 1 hr post-infection, the inoculum was aspirated and methionine-free medium. . .

DETD In order to determine whether the env gene product expressed by vCP83 and vCP87 was transported to the plasma **membrane** of infected cells, immunofluorescence experiments were performed as described previously (Taylor et al., 1990). Primary CEF monolayers were infected with. . . DETD . . . to challenge with feline leukemia virus

Time (weeks) relative to challenge

Group	Cat No.	-5	-2	0	+3	+6	+9	+12
	E[1] V[2]	EV	EV	EV	F[3] EV			
							FEV	FEV
vCP 93:	1	--	--	--	++	-++	+++	+++
Felv-A	2.	.	.		++	-++	+++	+++
	17	--	--	--	++	-++	+++	+++
	18	--	--	--	++	-++	+++	+++

*E = FeLV p27 antigen in plasma (ELISA)
V = infectious virus in plasma (virus isolation)
F = FeLV antigen in. . .

DETD The FHV-1 CO strain genomic DNA was completely digested with EcoRI and the fragment **M** (4470 bp) was excised from the agarose gel (Geneclean procedure) and cloned into vector pBS-SK+ digested with EcoRI and phosphatased. The resulting plasmid containing the FHV-1 EcoRI **M** fragment was designated pHFeM2. The FHV-1 EcoRI **M** fragment complete nucleotide sequence for both strands was obtained from several subclones of the FHV-1 EcoRI **M** fragment inserted into vector pBS-SK+, using the modified T7 enzyme Sequenase (U.S. Biochemical Corp.) (Tabor and Richardson, 1987). Standard dideoxynucleotide chain termination reactions (Sanger et al., 1977) were performed using double-stranded templates that had been denatured in 0.4 **M**-NaOH (Hattori and Sakaki, 1986). The M13 forward and reverse primers were used to obtain the initial sequence of each clone.. . .

DETD . . . the FHV-1 gD 5'-most region were confirmed by direct sequencing of pJCA071. Plasmid pJCA067 is a subclone of FHV-1 EcoRI **M** fragment. It has been generated as follows. Plasmid pHFeM2 was digested with BamHI and the 1850 bp BamHI-BamHI fragment was. . .

DETD Expression of the Hantaan virus G1 and G2 glycoproteins was accomplished by insertion of the **M** segment into the NYVAC and ALVAC vectors under the control of the entomopoxvirus 42 kDa promoter. The poxvirus expression cassette. . .

DETD A cDNA clone of the Hantaan virus **M** segment was derived as described by Schmaljohn et al. (1987) and provided by Dr. J. Dalrymple (Virology Division, U.S. Army. . . full sequence of the cDNA was presented previously by Schmaljohn et al. (1987). The 326 bp 5'-most region of the **M** segment coding sequence was derived using the plasmid pTZ19R containing the **M** segment cDNA as template and oligonucleotides HM5P (SEQ ID NO:335) (5'-ATGGGGATATGGAAGTGG-3') and HM3P (SEQ ID NO:336) (5'-CATGTTCCTTTCAAGTCAAC-3'). This 326 bp. . .

DETD The 3'-most 748 bp of the **M** segment coding sequence was derived by PCR using the cDNA clone contained in pTZ19R as template and oligonucleotides HMTS-5 (SEQ. . .

DETD The plasmid containing the **M**-specific cDNA clone in pTZ19R was used to transform GM48 (Dam$^-$) bacterial cells (BRL, Gaithersburg, Md.). Plasmid DNA derived from this. . . the 42 kDa promoter fused to the 5' most region of the coding sequence. The resultant plasmid containing the entire **M** segment expression cassette was designated as pBSHVM. The entire **M** segment cassette was excised from pBSHVM using restriction endonucleases HindIII and EcoRI. The 3508 bp derived fragment was

blunt end using the Klenow fragment of the **E**. coli in the presence of 2 mM dNTPs. The blunt ended fragment was inserted into pSD550to yield pHVMVC.

DETD . . . vP882. Recombinant virus was identified by in situ hybridization according to standard procedures (Piccini et al., 1987) using a radiolabeled **M**-specific DNA probe. Recombinant plaques were purified by 3 rounds of plaque purification and amplified for further analysis. Recombinant virus, vP882, contains the Hantaan **M** segment in the I4L locus of vaccinia virus. Replacement of the I4L open reading frame with the **M** segment cassette in the vP804 background creates a NYVAC- equivalent virus background (Tartaglia et al., 1992).

DETD The 3508 bp HindIII/EcoRI fragment derived from pBSHVM, containing the **M** segment cassette (above), was inserted into pC4I digested with HindIII and EcoRI. The plasmid pC4I was derived as follows. A. . .

DETD Insertion of the **M** segment cassette into pC4I yielded plasmid pC4HVM. The plasmid pC4HVM was linearized with SmaI for insertion of a 100 bp. . . pC4HVMVQ was digested with SmaI followed by a subsequent partial HindIII digestion to recover a 3.6 kb fragment containing the **M** segment cassette. This fragment was blunt-ended using the klenow fragment of the **E**. coli DNA polymerase in the presence of 2 mM dNTPs. This blunt-ended fragment was inserted into SmaI digested pSPCPC3L to. . .

DETD . . . identify and to purify the recombinant virus (as above; Piccini et al., 1987). The ALVAC-based recombinant containing the Hantaan virus **M** segment was designated as vCP114.

DETD . . . by linearization with XbaI followed by a partial HindIII digestion. This fragment was blunt-ended using the klenow fragment of the **E**. coli DNA polymerase in the presence of 2 mM dNTPs and then inserted into the SmaI site of pSD541L (defined. . .

DETD . . . in the ATI site and vP951 contains this cassette at the same locus, but by virtue of rescue with the **M** segment containing vP882, also contains the **M** segment in the I4L locus.

DETD The plasmid pBSHVM was linearized with SalI and blunt-ended using the Klenow fragment of the **E**. coli DNA polymerase in the presence of 2 mM dNTPs. This was ligated to the 1.4 kb XbaI/partial HindIII (Blunt-ended. . . from pBSHVS containing the Hantaan S segment expression cassette. The derived plasmid was designated as pBSHVMS. This plasmid contained the **M** and S cassettes in a head to head configuration. Plasmid pBSHVMS was linearized with XhoI, blunted with Klenow (as above),. . .

DETD . . . a 1.5 kb fragment containing the S segment expression cassette. This fragment was blunt-ended using the Klenow fragment of the **E**. coli DNA polymerase in the presence of 2 mM dNTPs and inserted into the SmaI site of pSPCP3L (defined in. . .

DETD . . . inoculated with NYVAC-based Hepatitis B virus (HBV) recombinants vP856, vP930, vP932 and vP975 (Example 13). vP856 expresses spsAg, the middle (**M**) form of the surface antigen. vP930 expresses lpsAg, the large (L) form of the surface antigen. vP932 expresses both spsAg. . .

DETD TABLE 34

AUSAB and CORAB
Rabbits
Analysis of sera of rabbits inoculated with NYVAC-based HBV recombinants expressing the middle (**M**) form of the surface antigen
(small pre S antigen), the large (L) form
of the surface antigen (large pre S antigen) and. . . 2 regions fused to the core antigen.

#	vP	HBV genes	week 1	2	3	4	5	6	7	8
AUSAB[a]										
A133										
	932									
		M + L	>512							
				>512						

202
352
13000
6500
3600
5400
A134
932
M + L 250 235 72 80 3900
561
800
218
A135
975
M + L + S/C
36 58 274
406
1300
646
436
268
A136
975
M + L + S/C
103 >512
127
136
13468
4968
3168
2768
CORAB[b]
A135
975
M + L + S/C
80 20 20 80 320 80 320
80
A136
975
M + L + S/C
20 5 5 5 80 80 320
80

Rabbits were inoculated with 10^8 pfu of the. . .
DETD TABLE 35

Pre-S2 ELISA
Rabbits
Analysis by ELISA of sera from rabbits inoculated with NYVAC-based HBV recombinants expressing the middle (**M**) form of the surface antigen, the large (L) form of the surface antigen and a fusion protein (S/C) consisting of the pre. . . 2 regions fused to the core antigen.

#	vP	HBV genes	week 1	2	3	4	5	6	7	8
A133										
932	**M** + L		0	0	29					
					35	474				
						602				
							358			
								419		
A134										
932	**M** + L		0	0	0	277				
						2017				
						3099				

500

A135
975 **M** + L + S/C
0 0 0 0 175
105
94 48

A136
975 **M** + L + S/C
0 0 0 0 2440
763
672
355

Rabbits were inoculated by the intramuscular (IM) route. . .
DETD TABLE 36

Pre-S1 ELISA
Rabbits
Analysis by ELISA test of sera from rabbits inoculated with NYVAC-based HBV recombinants expressing the middle (**M**) form and the large (L) form of the surface antigen and the preS 1 + 2/core fusion protein (S/C).

#	vP	HBV genes	week 0	2	4	5	6	8
A133								
	932	**M** + L	<10	<10	<10	<10	<10	<10
A134								
	932	**M** + L	<10	<10	<10	<10	17	<10
A135								
	975	**M** + L + S/C	<10	<10	15	40	<10	24
A136								
	975	**M** + L + S/C	15	16	17	117		
							52	49

Rabbits were inoculated by the intradermal (ID) route with 10^8. . .
DETD TABLE 37

Pre-S2 ELISA
Guinea Pigs
Analysis by ELISA of sera from guinea pigs inoculated with NYVAC-based HBV recombinants expressing the middle (**M**) form of the surface antigen, the large (L) form of the surface antigen and the preS 1 + 2/ core fusion protein (S/C).

#	vP	HBV genes	week 0	5	6
85	856	**M**	<10	<10	<10
86	856	**M**	<10	<10	<10
87	930	L	<10	46	35
88	930	L	<10	30	93
89	932	**M** + L	<10	39	<10
90	932	**M** + L	<10	33	19
91	975	**M** + L + S/C	<10	22	84
92	975	**M** + L + S/C	<10	53	269

Guinea pigs were inoculated by the SC route with 10^8 of the indicate
. . .
DETD TABLE 38

CORAB
Mice

Analysis of sera by CORAB test of mice inoculated with vaccinia recombinant vP975 expressing the HBV middle (**M**) form of the surface antigen, the large (L) form of the surface antigen and a fusion protein (S/C) consisting of the pre S. . . 2 regions fused to the core antigen

Group	vP	HBV genes	Week 1	2	3	4	5	6	7	8
D	975	**M** + L + S/C	-[a]	-	-	5	5	5	5	

Mice were inoculated by the IM route with 10^7. . .

DETD TABLE 39

Pre-S2 ELISA

Mice

Analysis by ELISA of sera from mice inoculated with NYVAC-based HBV recombinants expressing the middle (**M**) form of the surface antigen, the large (L) form of the surface antigen and the preS 1 + 2/core fusion protein (S/C).

	vP	HBV genes	week 0	5	6
Group A	856	**M**	<10	73	70
Group B	930	L	<10	93	112
Group C	932	**M** + L	<10	970	1146
Group D	975	**M** + L + S/C	<10	1054	1062

Groups of eight or twelve mice were inoculated by the IM route with 107 of. . .

DETD TABLE 40

Pre-S1 ELISA

Mice

Analysis by ELISA of sera from mice inoculated with NYVAC-based HBV recombinants expressing the middle (**M**) form of the surface antigen, the large (L) form of the surface antigen and the preS 1 + 2/core fusion protein (S/C).

	vP	HBV genes	week 0	5
Group B	930	L	60	244
Group C	932	**M** + L	66	125
Group D	975	**M** + L + S/C	63	1554

Groups of eight or twelve mice were inoculated by the IM route with 10^7 of the. . .

DETD . . . monolayers were either infected with parental virus, CPpp (ALVAC) or vP866 (NYVAC), or infected with vCP161 or vP1075 at an **m**.o.i. of 10 pfu/cell. Cells were infected, incubated in modified Eagle's medium (minus methionine) containing [^{35} S]-methionine (20 µCi/ml), lysed and. . .

DETD . . . by Makoff et al., 1989) for fragment C produced by papain digestion of native tetanus toxin as well as an **E**. coli produced recombinant fragment C which is identical to that encoded by vCP161 and vP1075.

DETD . . . days post-challenge. NYVAC-based pseudorabies virus recombinant viruses were all shown to reduce the effects of the virulent pseudorabies virus challenge (i.**e**. clinical signs and virus isolation) compared to the controls, with the gp50 expressing recombinant virus being the most efficacious. In. . .

DETD . . . receiving vP1015

A168 <1.3[d]

<1.3 <1.3 1.3[c]

					2.2	2.2	2.2	
A169	<1.3	1.6	1.6	1.6	3.1	3.1	2.5	
Animals receiving vP913								
A116	<1.3	<1.3	N.D[e]					
				<1.3	2.8	2.2	2.2	
A117	<1.3	<1.3	N.D.	<1.3	1.9	1.9	1.9	

[a] Day of inoculation with 8.0 $\log_{10}$ pfu of. . . highest dilution showing a 50% reduction in plaque number as compared to preinoculation serum.

[d] Lowest dilution tested was 1:20

[e] Not done

DETD CONSTRUCTION OF INSERTION VECTOR CONTAINING JAPANESE **ENCEPHALITIS VIRUS** (JEV) 15aaC, **prM**, **E**, NS21, NS2A

DETD . . . promoter, plasmid origin of replication and C5 flanking arms isolated. Plasmid JEVL14VC containing JEV cDNA encoding 15 amino acids C, **prM**, **E**, NS1, NS2A in a vaccinia virus donor plasmid (Mason et al., 1991) (nucleotides 337-4125, Konishi et al., 1991) was digested. . .

DETD CONSTRUCTION OF C5 INSERTION VECTOR CONTAINING JEV 15aaC, **prM**, **E**

DETD . . . annealed oligonucleotides SP131 (SEQ ID NO:382) and SP132 (SEQ ID NO:383) (containing a SphI sticky end, T nucleotide completing the **E** coding region, translation stop, a vaccinia early transcription termination signal (AT5AT; Yuen and Moss, 1987), a second translation stop, and XbaI sticky end) generating plasmid JEVCP5 which encodes 15 amino acids C, **prM** and **E** under the control of the H6 promoter between C5 flanking arms.

DETD JEVCP1 was transfected into ALVAC infected primary CEF cells to generate the canarypox recombinant vCP107 encoding 15 amino acids C, **prM**, **E**, NS1, NS2A. JEVCP5 was transfected into ALVAC infected primary CEF cells to generate the canarypox recombinant vCP140 encoding JEV 15 aa C, **prM** and **E**. ##STR41##

DETD Immunoprecipitation experiments were performed as described previously (Konishi et al., 1991). The **E** protein produced in vCP107 and vCP140 infected cells comigrates with the **E** protein produced by JEV-vaccinia recombinants which have been shown to produce an authentic **E** protein (Konishi et al., 1991). vCP107 produces an NS1 protein that comigrates with the NS1 protein produced by JEV-vaccinia recombinants. . .

DETD TABLE 48

Protective efficacy of TROVAC-NDV (vFP96) in SPF and commercial broiler chickens.

Bird Group	Dose	NDV HI GMT[d] Week 3	NDV HI GMT[d] Week 4	Percent Protection[e] NDV	Percent Protection[e] FP
Group 1[a]					
	2.0	<5	<5	70	100
	4.0	<5	<5	70	100

None. . . with prior history of vaccination with fowlpox virus

[c] Specific pathogen free birds

[d] Geometric mean titer of HI antibody

[e] Percent protection of birds after NDV or Fowpox challenge

DETD . . . centrifugation and resuspended in Assay Medium (RPMI 1640 containing 10% fetal bovine serum, 20 mM HEPES, 2 mM L-glutamine, 5×10^{-5} **M** 2-mercaptoethanol, 100 U/ml penicillin, and 100 µg/ml streptomycin). For memory CTL activity, the spleen cells from immunized mice were resuspended in Stimulation Medium (Minimum Essential Medium with Earle's salts containing 10% fetal bovine serum, 2 mM L-glutamine, 10^{-4} **M** 2-mercaptoethanol, 100 U/ml penicillin, and 100 µg/ml streptomycin) and stimulated in vitro in upright 25 cm^2 tissue culture flasks with. . . titrated effector cells in

96-well microtiter plates for a 4 hr ^{51}Cr release assay. Effector to target cell ratios (**E**:T) shown for the three assays were 100:1 (primary), 20:1 (memory), and 50:1 (secondary). Percent cytotoxicity was calculated as (experimental ^{51}. . .

DETD . . . and resuspended in the original volume of Cytotoxicity Medium, divided into two equal portions with or without complement (Rabbit Lo-Tox **M**, Cedarlane) and incubated at 37° C. for 45 min. The cells were then washed in Assay Medium and, based on. . .

DETD . . . apparent molecular masses of 160 kDa, 120 kDa, and 41 kDa, respectively. These are consistent with expression of the precursor **envelope** glycoprotein (160 kDa) and the proteolytically derived mature forms (120 kDa and 41 kDa).

DETD . . . digested pC5L to yield pC5HIV3BEEC. A 2.7 kb NruI/XbaI fragment from pBSHIV3BEECM was blunt-ended with the Klenow fragment of the **E**. coli DNA polymerase and inserted into NruI/SmaI digested pSPHAH6 to yield pHAHIV3BEEC.

DETD . . . by isolating the 2.1 kb NruI/XbaI fragment from pBSHIVMNT. This fragment was then blunt-ended with the Klenow fragment of the **E**. coli DNA polymerase in the presence of 2 mM dNTPs and inserted into pSPHAH6 digested with NruI and SmaI to. . .

DETD . . . Corp., Emeryville, Calif.). Investigation of surface immunofluorescence indicated that vCP138 and vP1035 infected cells contained HIV-1(MN) gp120 in the plasma **membrane**. Significantly, the surface staining of vCP138 and vP1035 infected cells was greatly enhanced compared to cells infected with recombinant viruses (i.**e**. vCP125, vCP124, vP1004, and vP1008) expressing gp160 or a non-anchored gp120. Results from immunoprecipitation analyses confirmed the expression of gp120. . .

DETD . . . precursor protein. Vero cell monolayers were either mock infected, infected with the parental virus or infected with vP969 at an **m**.o.i. of 10 PFU/cell. Following an hour adsorption period, the inoculum was aspirated and the cells were overlayed with 2 mls. . .

DETD An H6-promoted truncated HIV-1 **envelope** gene was then inserted into pHIVG4. This was accomplished by cloning the **E**. coli DNA polymerase I (Klenow fragment) filled-in 1,600 bp XhoI-NotI fragment of pHIVE10, containing an H6-promoted truncated HIV-1 **envelope** gene, into the filled-in BamHI site of pHIVG4. The plasmid generated by this manipulation is called pHIVGE11.

DETD . . . New Haven, Conn.). The plasmid pBSHIV3BCDT1 contains an H6 promoted cassette to express a severely truncated form of the HIV-1(IIIB) **envelope** (amino acid 1 to 447; Ratner et al., 1985). Expression of this cassette was evaluated to eliminate CD4 binding while. . .

DETD An H6-promoted truncated HIV-1 **envelope** gene was then inserted into pHIVG7. This was accomplished by cloning the **E**. coli DNA polymerase I (Klenow fragment) filled-in 1,600 bp XhoI-NotI fragment of pHIVE10 (defined in Example 95), containing an H6-promoted truncated HIV-1 **envelope** gene, into the filled-in BamHI site of pHIVG7. The plasmid generated by this manipulation is called pHIVGE12.

DETD The H6-promoted HIV-1(MN) **envelope** (gp120) gene was then inserted into pHIVGE14. This was accomplished by cloning the oligonucleotides, HIVL29 (SEQ ID NO:421) (5'-GGCCGCAAC-3') and. . .

DETD The H6-promoted **envelope** (gp120) gene and the I3L-promoted gag and pol genes were then inserted into a vaccinia virus insertion vector. This was. . .

DETD . . . by the gp160 gene. This was accomplished by cloning the 2,600 bp NruI-NotI fragment of pH6HMNE, containing the entire HIV-1(MN) **envelope** (gp160) gene, into the 8,000 bp partial NruI-NotI fragment of pHIVGE16. The plasmid generated by this manipulation is called pHIVGE19.

DETD . . . gene products. CEF cell monolayers were either mock infected, infected with the parental virus or infected with vCP117 at an **m**.o.i. of 10 PFU/cell. Following an hour adsorption period, the inoculum was aspirated and the cells were overlayed with 2 mls. . .

DETD . . . by the gp160 gene. This was accomplished by cloning the 2,600 bp NruI-NotI fragment of pH6HMNE, containing the entire HIV-1(MN) **envelope** (gp160) gene, into the 9,800 bp NruI-NotI fragment of

pHVGB19. The plasmid generated by this manipulation is called pHVGB19.

DETD . . . gene products. CEF cell monolayers were either mock infected, infected with the parental virus or infected with vCP130 at an **m**.o.i. of 10 PFU/cell. Following an hour adsorption period, the inoculum was aspirated and the cells were overlayed with 2 mls. . .

DETD . . . gag-pol and env genes would also produce such particles. Furthermore, if these ALVAC-based recombinants were used to infect non-avian cells (i.**e**. Vero, MRC-5, etc.) then HIV-1 virus-like particles could be purified without any poxvirus virion contaminants.

DETD . . . evaluate particle formation using Vero cells infected with vCP156, the following experiment was performed. Vero cells were infected at an **m**.o.i. of approximately 5 pfu/cell. After a 24 hr infection period, the supernatant was harvested and clarified by centrifugation at 2000. . . With the size exclusion noted above, the p24 would have passed through unless it was in a higher structural configuration (i.**e**. virus-like particles). Therefore, these results strongly suggest that HIV-1 virus-like particles containing the gp120 **envelope** component are produced in vCP156 infected cells.

DETD . . . gene products. Vero cell monolayers were either mock infected, infected with the parental virus or infected with vP1045 at an **m**.o.i. of 10 PFU/cell. Following an hour adsorption period, the inoculum was aspirated and the cells were overlayed with 2 mls. . .

DETD . . . gene products. CEF cell monolayers were either mock infected, infected with the parental virus or infected with vCP153 at an **m**.o.i. of 10 PFU/cell. Following an hour adsorption period, the inoculum was aspirated and the cells were overlayed with 2 mls. . .

DETD . . . precursor proteins. Vero cell monolayers were either mock infected, infected with the parental virus or infected with vP948 at an **m**.o.i. of 10 PFU/cell. Following an hour adsorption period, the inoculum was aspirated and the cells were overlayed with 2 mls. . .

DETD Macaque sera from SIV seropositive individuals specifically precipitated the SIV gag precursor protein and the **envelope** glycoprotein from vP948 infected cells, but did not precipitate SIV-specific proteins from mock infected cells.

DETD The plasmid, pSIVEMVC, contains the H6-promoted SIV_{MAC142} **envelope** gene (in vitro selected truncated version). The region of the **envelope** gene containing the premature termination codon was cloned into pBSK+. This was accomplished by cloning the 1,120 bp ClaI-BamHI fragment. . .

DETD At day 56 (i.**e**. 28 days after the second injection) protective titers were achieved in 0/3 of Group A, 2/3 of Group B and. . .

DETD . . . $10^3.5$

		<0.1	<0.1	<0.1	<0.1	0.2
3	$10^3.5$					
		<0.1	<0.1	<0.1	<0.1	<0.1
4	$10^3.5$					
		<0.1	<0.1	<0.1	<0.1	<0.1
	G.M.T.	<0.1	<0.1	<0.1	<0.1	<0.1
6	$10^4.5$					
		<0.1	<0.1	<0.1	<0.1	<0.1
7	$10^4.5$					
		<0.1	<0.1	<0.1	2.4	1.9
10	$10^4.5$					
		<0.1	<0.1	<0.1	1.6	1.1
	G.M.T.	<0.1	<0.1	0.1	0.58	0.47
11	$10^5.5$					
		<0.1	<0.1	1.0	3.2	4.3
13	$10^5.5$					
		<0.1	<0.1	0.3	6.0	8.8
14	$10^5.5$					
		<0.1	<0.1.	. .	0.3	3.7
21	$10^5.5$					
		<0.1	<0.1	0.2	2.6	3.9
23	$10^5.5$					
		<0.1	<0.1	<0.1	1.7	4.2

25	$10^{-.5}$					
		<0.1	<0.1	<0.1	0.6	0.9
	G.**M**.T.	<0.1	<0.1	0.16	1.9	4.4*
2	HDC	<0.1	<0.1	0.8	7.1	7.2
5	HDC	<0.1	<0.1	9.9	12.8	18.7
8.	. .	7.7	20.7			
19	HDC	<0.1	<0.1	2.6	9.9	9.1
22	HDC	<0.1	<0.1	1.4	8.6	6.6
24	HDC	<0.1	<0.1	0.8	5.8	4.7
	G.**M**.T.	<0.1	<0.1	2.96	9.0	11.5*

*p = 0.007 student t test

DETD PROTECTION AGAINST JAPANESE **ENCEPHALITIS VIRUS** BY NYVAC-JEV RECOMBINANTS (vP908, vP923)

DETD Using NYVAC-JEV recombinants, protection against Japanese **Encephalitis virus** was provided. NYVAC vP866, NYVAC recombinants vP908 and vP923, and vaccinia recombinants vP555 and vP829 were produced as described herein.

DETD . . . positioned behind the early/late H6 promoter. Recombinant vP908 (and vP555; Mason et al., 1991) includes the putative 15 amino acid **signal sequence** preceding the N-terminus of **prM**, **prM**, **E**, NS1 and NS2A. Recombinant vP923 (and vP828; Konishi et al., 1991) encodes the putative **signal sequence** of **prM**, **prM**, and **E**.

DETD Synthesis of **E** and NS1 by Recombinant Vaccinia Viruses. Immunoprecipitation of the **E** or NS1 gene was performed using a monoclonal antibody specific for **E** or NS1. Proteins reactive with the **E** MAb were synthesized in cells infected with vP555, vP908 and vP923, and proteins reactive with NS1 MAb were synthesized in. . . cells infected with vP555 and vP908 but not in cells infected with vP923. vP555 infected cells produced correct forms of **E** and NS1 inside and outside of the cell. The proteins produced by vP908 and vP923 were identical in size to those produced by vP555. For both **E** and NS1, the extracellular forms migrated slower than the intracellular forms in SDS-PAGE, consistent with maturation of the N-linked glycans. . . the JEV genome (Mason et al., 1987). Immunoprecipitates prepared from radiolabeled vaccinia recombinant infected cells using a MAb specific for **M** (and **prM**) revealed that **prM** was synthesized in cells infected with vP908 and vP923.

DETD The immune response to **E** correlated well with the results of the NEUT and HAI tests. The RIP response to **E** observed in swine immunized with vP923 on day 35 was higher than the RIP response to **E** in swine immunized with vP908, whereas the HAI titers on day 35 were equivalent in the two groups. However, NEUT. . . be induced but the quantitative aspects of the RIP analysis was not further validated. Weak RIP responses of sera to **E** on day seven in spite of relatively high NEUT antibody titers could be explained by IgM antibody early after immunization.. . .

DETD . . . sera collected 20 days post-challenge for antibodies against JEV. The swine vaccinated with vP908 or vP923 had higher responses to **E** than those inoculated with PBS or vP866, indicating that the antibody reactivity to **E** that was present before challenge was boosted by JEV infection. Reactions to NS3 and NS5, JEV proteins which were not. . .

DETD TABLE 52

Immunization and JEV challenge in mice

Immunizing Virus[a]	JEV Genes Expressed	NEUT[b]	HAI[c]	Survival[d]
vP829	**prM**, **E**	1:320	1:80	10/10 (100%)
vP866	None	<1:10	<1:10	0/12 (0%)
vP908	**prM**, **E**,	1:320	1:80	11/12 (92%)

vP923	**prM, E**	1:320	1:80	12/12 (100%)

[a] Vaccinia recombinant virus used for immunizing groups of 4week old mice.

[b] Serum dilution yielding. . .

DETD . . . recombinants have also been shown to elicit measles virus neutralizing antibodies in rabbits and protection against pseudorabies virus and Japanese **encephalitis virus** challenge in swine. The highly attenuated NYVAC strain confers safety advantages with human and veterinary applications (Tartaglia et al., 1990).. . .

DETD . . . skin); c) absence of testicular inflammation (nude mice); d) greatly reduced virulence (intracranial challenge, both three-week old and newborn mice); **e**) greatly reduced pathogenicity and failure to disseminate in immunodeficient subjects (nude and cyclophosphamide treated mice); and f) dramatically reduced ability. . .

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. . . claimed in claim 2 wherein the non-vaccinia source is selected from the group consisting of rabies virus, Hepatitis B virus, **yellow fever virus**, **Dengue** virus, pseudorabies virus, Epstein-Barr virus, herpes simplex virus, simian immunodeficiency virus, equine herpes virus, bovine herpes virus, bovine viral diarrhea. . .

6. A recombinant vaccinia virus as claimed in claim 3, wherein the non-vaccinia source is **yellow fever virus** and the recombinant vaccinia virus is vP766, vP764, vP869, vP729, vP725, vP997, or vP984.

7. A recombinant vaccinia virus as claimed in claim 3, wherein the non-vaccinia source is **Dengue** virus and the recombinant vaccinia virus is vP867, vP962 or vP955.

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L2            106 S E3
L3              2 S L2 AND FLAVIVIR?

     FILE 'MEDLINE' ENTERED AT 16:01:13 ON 30 APR 2004
                E CHANG G J/AU
L4             49 S E3
L5             29 S L4 AND (FLAVIVIR? OR YELLOW OR DENGUE OR ENCEPHALITIS)
L6              9 S L5 AND (PRM OR E OR PRM/M OR PRM/E)

     FILE 'USPATFULL' ENTERED AT 16:06:37 ON 30 APR 2004
L7           3470 S (FLAVIVIR? OR YELLOW FEVER VIR? OR DENGUE OR ENCEPHALITIS VIR
L8             79 S L7 AND (PRM OR PREMEMBRANE)
L9             79 S L8 AND (E OR ENVELOPE)
L10            79 S L9 AND (M OR MEMBRANE)
L11            43 S L10 AND (SIGNAL SEQUENCE)
L12             5 S L11 AND KOZAK
L13            38 S L11 NOT L12
L14            15 S L13 AND AY<1999

=> s l7 and (kozak or ribosome binding site)
          5840 KOZAK
         18689 RIBOSOME
        221937 BINDING
        339909 SITE
         10959 RIBOSOME BINDING SITE
                (RIBOSOME(W)BINDING(W)SITE)
L15          805 L7 AND (KOZAK OR RIBOSOME BINDING SITE)

=> s l15 and kozak/clm
            38 KOZAK/CLM
L16          2 L15 AND KOZAK/CLM

=> d l16,cbib,1-2

L16  ANSWER 1 OF 2  USPATFULL on STN
2003:30900 Nucleic acid vaccines for prevention of flavivirus infection.
    Chang, Gwong-Jen J., Fort Collins, CO, UNITED STATES
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    US 2005022649 A1 20050130
    APPLICATION: US 2001-826115  A1  20010404 (9)
    PRIORITY: US 1998-87908P  19980604 (60)
    DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L16  ANSWER 2 OF 2  USPATFULL on STN
2002:148552 Ribosome display.
    Osbourn, Jane, Cambridge, UNITED KINGDOM
    Holet, Thor L., Frederikssund, DENMARK
    US 2002076692 A1 20020620
    APPLICATION: US 2001-817661  A1  20010326 (9)
    PRIORITY: US 2000-193802P  20000331 (60)
    DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.


=> s l15 and (kozak ribosome binding site? or kozak concensus sequenc?)
          5840 KOZAK
         18689 RIBOSOME
        221937 BINDING
        422889 SITE?
             5 KOZAK RIBOSOME BINDING SITE?
                 (KOZAK(W)RIBOSOME(W)BINDING(W)SITE?)
          5840 KOZAK
           517 CONCENSUS
        666705 SEQUENC?
             5 KOZAK CONCENSUS SEQUENC?
                 (KOZAK(W)CONCENSUS(W)SEQUENC?)
L17          0 L15 AND (KOZAK RIBOSOME BINDING SITE? OR KOZAK CONCENSUS SEQUENC
               ?)

=> d his

     (FILE 'HOME' ENTERED AT 15:58:13 ON 30 APR 2004)

     FILE 'USPATFULL' ENTERED AT 15:59:07 ON 30 APR 2004
                E CHANG GWONG JEN/IN
L1            1 S E4

     FILE 'WPIDS' ENTERED AT 16:00:09 ON 30 APR 2004
                E CHANG G J/IN
L2          106 S E3
L3            2 S L2 AND FLAVIVIR?

     FILE 'MEDLINE' ENTERED AT 16:01:13 ON 30 APR 2004
                E CHANG G J/AU
L4           49 S E3
L5           29 S L4 AND (FLAVIVIR? OR YELLOW OR DENGUE OR ENCEPHALITIS)
L6            9 S L5 AND (PRM OR E OR PRM/M OR PRM/E)

     FILE 'USPATFULL' ENTERED AT 16:06:37 ON 30 APR 2004
L7         3470 S (FLAVIVIR? OR YELLOW FEVER VIR? OR DENGUE OR ENCEPHALITIS VIR
L8           79 S L7 AND (PRM OR PREMEMBRANE)
L9           79 S L8 AND (E OR ENVELOPE)
L10          79 S L9 AND (M OR MEMBRANE)
L11          43 S L10 AND (SIGNAL SEQUENCE)
L12           5 S L11 AND KOZAK
L13          38 S L11 NOT L12
L14          15 S L13 AND AY<1999
L15         805 S L7 AND (KOZAK OR RIBOSOME BINDING SITE)
L16           2 S L15 AND KOZAK/CLM
L17           0 S L15 AND (KOZAK RIBOSOME BINDING SITE? OR KOZAK CONCENSUS SEQU

=> s l15 and (CMV or cytomegalovirus)
         16857 CMV
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          19250 CYTOMEGALOVIRUS
L18         716 L15 AND (CMV OR CYTOMEGALOVIRUS)

=> s l18 and (IE or immediate-early promoter?)
         40119 IE
        166841 IMMEDIATE
        207797 EARLY
         89523 PROMOTER?
          3068 IMMEDIATE-EARLY PROMOTER?
                 (IMMEDIATE(W)EARLY(W)PROMOTER?)
L19         191 L18 AND (IE OR IMMEDIATE-EARLY PROMOTER?)

=> ss l19 and (CMV/clm or cytomegalovirus/clm)
           818 CMV/CLM
          1155 CYTOMEGALOVIRUS/CLM
L20          30 L19 AND (CMV/CLM OR CYTOMEGALOVIRUS/CLM)

=> s l20 and (IE/clm or immediate-early/clm)
           920 IE/CLM
          9057 IMMEDIATE/CLM
          4941 EARLY/CLM
           269 IMMEDIATE-EARLY/CLM
                 ((IMMEDIATE(W)EARLY)/CLM)
L21            2 L20 AND (IE/CLM OR IMMEDIATE-EARLY/CLM)

=> d l21,cbib,1-2

L21  ANSWER 1 OF 2  USPATFULL on STN
2003:213687 Infectious clones.
    Sanchez, Luis Enjuanes, Madrid, SPAIN
    US 2003148325 A1 20030807
    APPLICATION: US 2002-238786  A1  20020911 (10)
    PRIORITY: ES 1999-2673  19991203
    DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L21  ANSWER 2 OF 2  USPATFULL on STN
2003:30900 Nucleic acid vaccines for prevention of flavivirus infection.
    Chang, Gwong-Jen J., Fort Collins, CO, UNITED STATES
    US 2003022849 A1 20030130
    APPLICATION: US 2001-826115  A1  20010404 (9)
    PRIORITY: US 1998-87908P  19980604 (60)
    DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.


=> d his

     (FILE 'HOME' ENTERED AT 15:58:13 ON 30 APR 2004)

     FILE 'USPATFULL' ENTERED AT 15:59:07 ON 30 APR 2004
                E CHANG GWONG JEN/IN
L1              1 S E4

     FILE 'WPIDS' ENTERED AT 16:00:09 ON 30 APR 2004
                E CHANG G J/IN
L2            106 S E3
L3              2 S L2 AND FLAVIVIR?

     FILE 'MEDLINE' ENTERED AT 16:01:13 ON 30 APR 2004
                E CHANG G J/AU
L4             49 S E3
L5             29 S L4 AND (FLAVIVIR? OR YELLOW OR DENGUE OR ENCEPHALITIS)
L6              9 S L5 AND (PRM OR E OR PRM/M OR PRM/E)

     FILE 'USPATFULL' ENTERED AT 16:06:37 ON 30 APR 2004
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L7          3470 S (FLAVIVIR? OR YELLOW FEVER VIR? OR DENGUE OR ENCEPHALITIS VIR
L8            79 S L7 AND (PRM OR PREMEMBRANE)
L9            79 S L8 AND (E OR ENVELOPE)
L10           79 S L9 AND (M OR MEMBRANE)
L11           43 S L10 AND (SIGNAL SEQUENCE)
L12            5 S L11 AND KOZAK
L13           38 S L11 NOT L12
L14           15 S L13 AND AY<1999
L15          805 S L7 AND (KOZAK OR RIBOSOME BINDING SITE)
L16            2 S L15 AND KOZAK/CLM
L17            0 S L15 AND (KOZAK RIBOSOME BINDING SITE? OR KOZAK CONCENSUS SEQU
L18          716 S L15 AND (CMV OR CYTOMEGALOVIRUS)
L19          191 S L18 AND (IE OR IMMEDIATE-EARLY PROMOTER?)
L20           30 SS L19 AND (CMV/CLM OR CYTOMEGALOVIRUS/CLM)
L21            2 S L20 AND (IE/CLM OR IMMEDIATE-EARLY/CLM)

=> s l20 not l21
L22           28 L20 NOT L21

=> d l22,ti,1-28

L22  ANSWER 1 OF 28  USPATFULL on STN
TI     Respiratory syncytial virus vaccines expressing protective antigens from
       promotor- proximal genes

L22  ANSWER 2 OF 28  USPATFULL on STN
TI     Expression of HIV polypeptides and production of virus-like particles

L22  ANSWER 3 OF 28  USPATFULL on STN
TI     Polynucleotides encoding antigenic HIV type C polypeptides, polypeptides
       and uses thereof

L22  ANSWER 4 OF 28  USPATFULL on STN
TI     Polynucleotides encoding antigenic HIV type B and/or type C
       polypeptides, polypeptides and uses thereof

L22  ANSWER 5 OF 28  USPATFULL on STN
TI     Polynucleotides encoding antigenic HIV type B polypeptides, polypeptides
       and uses thereof

L22  ANSWER 6 OF 28  USPATFULL on STN
TI     Polynucleotides encoding antigenic HIV type B polypeptides, polypeptides
       and uses thereof

L22  ANSWER 7 OF 28  USPATFULL on STN
TI     Expression of HIV polypeptides and production of virus-like particles

L22  ANSWER 8 OF 28  USPATFULL on STN
TI     Polynucleotides encoding antigenic HIV type C polypeptides, polypeptides
       and uses thereof

L22  ANSWER 9 OF 28  USPATFULL on STN
TI     Host cells containing multiple integrating vectors

L22  ANSWER 10 OF 28  USPATFULL on STN
TI     Lentiviral vectors

L22  ANSWER 11 OF 28  USPATFULL on STN
TI     Methods and compositions useful for stimulating an immune response

L22  ANSWER 12 OF 28  USPATFULL on STN
TI     Multifunctional molecular complexes for gene transfer to cells

L22  ANSWER 13 OF 28  USPATFULL on STN
TI     Recombinant alphavirus-based vectors with reduced inhibition of cellular
       macromolecular synthesis
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L22 ANSWER 14 OF 28 USPATFULL on STN
TI Recombinant alphavirus-based vectors with reduced inhibition of cellular macromolecular synthesis

L22 ANSWER 15 OF 28 USPATFULL on STN
TI Alphavirus structural protein expression cassettes

L22 ANSWER 16 OF 28 USPATFULL on STN
TI Recombinant alphavirus-based vectors with reduced inhibition of cellular macromolecular synthesis

L22 ANSWER 17 OF 28 USPATFULL on STN
TI COMPOSITIONS AND METHODS FOR TREATING INFECTIONS USING CATIONIC PEPTIDES ALONE OR IN COMBINATION WITH ANTIBIOTICS

L22 ANSWER 18 OF 28 USPATFULL on STN
TI Dendritic marker-expressing macrophage cultures and methods for reactivating latent HCMV

L22 ANSWER 19 OF 28 USPATFULL on STN
TI DNA vaccines against tick-borne **flaviviruses**

L22 ANSWER 20 OF 28 USPATFULL on STN
TI Dendritic-marker expressing macrophage cultures and methods of reactivating latent virus

L22 ANSWER 21 OF 28 USPATFULL on STN
TI Lentiviral vectors

L22 ANSWER 22 OF 28 USPATFULL on STN
TI Compositions and methods for delivery of genetic material

L22 ANSWER 23 OF 28 USPATFULL on STN
TI Chimeric hepatitis B/hepatitis C virus vaccine

L22 ANSWER 24 OF 28 USPATFULL on STN
TI Method for stimulating an immune response utilizing recombinant alphavirus particles

L22 ANSWER 25 OF 28 USPATFULL on STN
TI Compositions and methods for delivery of genetic material

L22 ANSWER 26 OF 28 USPATFULL on STN
TI Compositions and methods for delivery of genetic material

L22 ANSWER 27 OF 28 USPATFULL on STN
TI Genetic immunization

L22 ANSWER 28 OF 28 USPATFULL on STN
TI Genetic immunization

=> s l20 and ay<1999
2806970 AY<1999
L23 10 L20 AND AY<1999

=> d l23,cbib,ab,clm,1-10

L23 ANSWER 1 OF 10 USPATFULL on STN
2002:61226 COMPOSITIONS AND METHODS FOR TREATING INFECTIONS USING CATIONIC PEPTIDES ALONE OR IN COMBINATION WITH ANTIBIOTICS.
KRIEGER, TIMOTHY J., RICHMOND, CANADA
TAYLOR, ROBERT, SURREY, CANADA
ERFLE, DOUGLAS, VANCOUVER, CANADA
FRASER, JANET R., VANCOUVER, CANADA

WEST, MICHAEL H.F., VANCOUVER, CANADA
MCNICHOL, PATRICIA J., COQUITLAM, CANADA
US 2002035061 A1 20020321
APPLICATION: US 1998-30619 A1 19980227 (9) <--
PRIORITY: US 1997-40649P 19970310 (60)
US 1997-60099P 19970926 (60)
US 1996-24754P 19960821 (60)
US 1997-34949P 19970113 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Compositions and methods for treating infections, especially bacterial infections, are provided. Indolicidin peptide analogues containing at least two basic amino acids are prepared. The analogues are administered as modified peptides, preferably containing photo-oxidized solubilizer.
CLM What is claimed is:

1. An indolicidin analogue, comprising 8 to 25 amino acids and containing the formula: RXZXXZXB wherein Z is proline or valine; X is a hydrophobic residue; and B is a basic amino acid.

2. An indolicidin analogue, comprising 8 to 25 amino acids and containing the formula: BXZXXZXB wherein Z is proline or valine; X is a hydrophobic residue; B is a basic amino acid; and wherein at least one Z is valine.

3. An indolicidin analogue, comprising 10 to 25 amino acids and containing the formula: BBBXZXXZXB or BXXBZBXBXZB wherein Z is proline or valine; X is a hydrophobic residue; and B is a basic amino acid.

4. An indolicidin analogue, comprising 17 to 25 amino acids and containing the formula: $BXZXXZXBBB_n(AA)_{nMILBBAGS}$ wherein Z is proline or valine; X is a hydrophobic residue; B is a basic amino acid; AA is any amino acid, and n is 0 or 1.

5. An indolicidin analogue, comprising 10 to 25 amino acids and containing the formula: $BXZXXZXBB(AA)_{nM}$ wherein Z is proline or valine; X is a hydrophobic residue; B is a basic amino acid; AA is any amino acid, and n is 0 or 1.

6. An indolicidin analogue, comprising 8 to 25 amino acids and containing the formula: $LBB_{nXZnXXZnXRK}$ wherein Z is proline or valine; X is a hydrophobic residue; B is a basic amino acid; and n is 0 or 1.

7. An indolicidin analogue, comprising 10 to 25 amino acids and containing the formula: $LK_{nXZXXZXRRK}$ wherein Z is proline or valine; X is a hydrophobic residue; and n is 0 or 1.

8. An indolicidin analogue, comprising 11 to 25 amino acids and containing the formula: BBXZXXZXBBB wherein Z is proline or valine; X is a hydrophobic residue; B is a basic amino acid; and wherein at least two X residues are phenylalanine.

9. An indolicidin analogue, comprising 11 to 25 amino acids and containing the formula: BBXZXXZXBBB wherein Z is proline or valine; X is a hydrophobic residue; B is a basic amino acid; and wherein at least two X residues are tyrosine.

10. The analogues according to any one of claims 1 and 3-7 wherein Z is proline, X is tryptophan and B is arginine or lysine.

11. An indolicin analogue selected from the group consisting of:

11B7 Ile Leu Arg Trp Pro Trp Trp Pro Trp Arg Arg Lys:

11B17 Ile Leu Arg Trp Pro Trp Trp Pro Trp Arg Arg Lys Met Ile Leu

Lys Lys Ala Gly Ser:

11CNR Lys Arg Arg Trp Pro Trp Trp Pro Trp Lys Lys Leu Ile;

11D18 Trp Arg Ile Trp Lys Pro Lys Trp Arg Leu Pro Lys Trp:

11F4 Ile Leu Arg Trp Val Trp Trp Val Trp Arg Arg Lys;

11FS Ile Leu Arg Arg Trp Val Trp Trp Val Trp Arg Arg Lys:

11G25 Leu Arg Trp Trp Trp Pro Trp Arg Arg Lys:

11H01 Ala Leu Arg Trp Pro Trp Trp Pro Trp Arg Arg Lys:

11H05 Ile Leu Arg Trp Ala Trp Trp Pro Trp Arg Arg Lys: and

11J02 Trp Arg Trp Trp Lys Pro Lys Trp Arg Trp Pro Lys Trp:

12. An indolicidin analogue selected from the group consisting of:

MBI 11A2: Ile Leu Lys Lys Ile Pro Ile Ile Pro Ile Arg Arg Lys;

MBI 11A3: Ile Leu Lys Lys Tyr Pro Tyr Tyr Pro Tyr Arg Arg Lys:

MBI 11A5: Ile Leo Lys Lys Tyr Pro Trp Tyr Pro Trp Arg Arg Lys;

MBI 11A6: Ile Leu Lys Lys Phe Pro Trp Phe Pro Trp Arg Arg Lys;

MBI 11A7: Ile Leu Lys Lys Phe Pro Phe Trp Pro Trp Arg Arg Lys:

MBI 11A8: Ile Leu Arg Tyr Val Tyr Tyr Val Tyr Arg Arg Lys;

MBI 11A9: Ile Leu Arq Trp Pro Trp Trp Pro Trp Trp Pro Trp Arg Arg Lys:

MBI 11A10: Trp Trp Arg Trp Pro Trp Trp Pro Trp Arg Arg Lys:

MBI 11B1: Ile Leu Arg Arg Trp Pro Trp Trp Pro Trp Arg Arg Lys:

MBI 11B2: Ile Leu Arg Arg Trp Pro Trp Trp Pro Trp Arg Lys;

MBI 1103: Ile Leu Lys Trp Pro Trp Trp Pro Trp Arg Arg Lys:

MBI 11B4: Ile Leu Lys Lys Trp Pro Trp Trp Pro Trp Arg Lys:

MBI 11B5: Ile Leu Lys Trp Pro Trp Trp Pro Trp Arg Lys:

MBI 11B7R: Lys Arg Arg Trp Pro Trp Trp Pro Trp Arg Leu Ile:

MBI 11B16: Ile Leu Arg Trp Pro Trp Trp Pro Trp Arg Arg Lys Ile Met Ile Leu Lys Lys Ala Gly Ser:

MBI 11B18: Ile Leu Arg Trp Pro Trp Trp Pro Trp Arg Arg Lys Asp Met Ile Leu Lys Lys Ala Gly Ser;

MBI 11019: Ile Leu Arg Trp Pro Trp Arg Arg Trp Pro Trp Arg Arg Lys:

MBI 11B20: Ile Leu Arg Trp Pro Trp Trp Pro Trp Arg Arg Lys Met Ile Leu Arg Trp Pro Trp Trp Pro Trp Arg Arg Lys Met Ala Ala

MBI 11D5: Ile Leu Lys Lys Trp Pro Trp Trp Pro Trp Arg Arg Met Ile Leu Lys Lys Ala Gly Ser;

MBI 11D6: Ile Leu Lys Lys Trp Pro Trp Trp Pro Trp Arg Arg Ile Met

Ile Leu Lys Lys Ala Gly Ser:

MBI 11D12: Ile Leu Lys Lys Trp Pro Trp Trp Pro Trp Arg Arg Met:

MBI 11D13: Ile Leu Lys Lys Trp Pro Trp Trp Pro Irp Arg Arg Ile Met:

MBI 11D4: Ile Leu Lys Lys Trp Trp Trp Pro Trp Arg Lys:

MBI 11D15: Ile Leu Lys Lys Trp Pro Trp Trp Trp Arg Lys:

MDI 11F1: Ile Leu Lys Lys Trp Val Trp Trp Val Trp Arg Arg Lys:

MDI 11F2: Ile Leu Lys Lys Trp Pro Trp Trp Val Trp Arg Arg Lys:

MDI 11F3: Ile Leu Lys Lys Trp Val Trp Trp Pro Trp Arg Arg Lys;

MDI 11F4R: Lys Arg Arg Trp Val Trp Trp Val Trp Arg Leu Ile:

MDI 11F6: Ile Leu Arg Trp Trp Val Trp Trp Val Trp Trp Arg Arg Lys:

MDI 11G26: Leu Arg Trp Pro Trp Trp Pro Trp:

MBI 11G28: Arg Trp Trp Trp Pro Trp Arg Arg Lys:

MBI 11J01: Arg Arg Ile Trp Lys Pro Lys Trp Arg Leu Pro Lys Arg:

13. An indolicidin analogue selected from the group consisting of:

MBI 11A4: Ile Leu Lys Lys Irp Pro Trp Pro Trp Arg Arg Lys;

MBI 11B8: Ile Leu Trp Pro Trp Trp Pro Trp Arg Arg Lys:

MBI 11D1: Leu Lys Lys Trp Pro Trp Trp Pro Trp Arg Arg Lys;

MBI 11D3: Pro Trp Trp Pro Trp Arg Arg Lys:

MBI 11D4: Ile Leu Lys Lys Trp Pro Trp Trp Pro Trp Arg Arg Lys Met.

Ile Leu Lys Lys Ala Gly Ser:

MBI 11D9: Trp Trp Pro Trp Arg Arg Lys:

MBI 11D10: Ile Leu Lys Lys Trp Pro Trp.

MBI 11D11: Ile Leu Lys Lys Trp Pro Trp Trp Pro Trp Arg Arg Lys Met:

MBI 11G2: Ile Lys Lys Irp Pro Trp Trp Pro Trp Arg Arg Lys:

MBI 11G3: Ile Leu Lys Lys Pro Trp Trp Pro Trp Arg Arg Lys;

MBI 11G4: Ile Leu Lys Lys Trp Trp Trp Pro Trp Arg Arg Lys:

MBI 11G5: Ile Leu Lys Lys Trp Pro Trp Trp Trp Arg Arg Lys;

MBI 11G6: Ile Leu Lys Lys Trp Pro Trp Trp Pro Arg Arg Lys:

MBL 11G13: Ile Leu Lys Lys Trp Pro Irp Trp Pro Trp Lys:

MBI 11G14: Ile Leu Lys Lys Trp Pro Trp Irp Pro Trp Arg:

MBI 11G16: Trp Pro Trp Trp Pro Trp Arg Arg Lys:

MBI 11G24: Leu Trp Pro Irp Trp Pro Trp Arg Arg

Lys.

MBT 11G27: Trp Pro Trp Trp Pro Trp Arg Arg Lys;

14. An indolicidin analogue selected from the group consisting of:

MBI 11H2: Ile Ala Arg Trp Pro Trp Trp Pro Trp Arg Arg Lys:

MBI 11H3: Ile Leu Ala Trp Pro Trp Trp Pro Trp Arg Arg Lys:

MBI 11H4: Ile Leu Arg Ala Pro Trp Trp Pro Trp Arg Arg Lys:

MBI 11H6: Ile Leu Arg Trp Pro Ala Trp Pro Trp Arg Arg Lys:

MBI 11H7: Ile Leu Arg Trp Pro Trp Ala Pro Trp Arg Arg Lys:

MEI 11H8: Ile Leu Arg Trp Pro Trp Trp Ala Trp Arg Arg Lys:

MBI 11H9: Ile Leu Arg Trp Pro Trp Trp Pro Ala Arg Arg Lys;

MEI 11H10: Ile Leu Arg Trp Pro Trp Trp Pro Trp Ala Arg Lys:

MBI 11H11: Ile Leu Arg Trp Pro Trp Trp Pro Trp Arg Ala Lys:

MBI 11H12: Ile Leu Arg Trp Pro Trp Trp Pro Trp Arg Arg Ala:

15. The indolicidin analogue according to any one of claims 1-14, wherein two or more analogues are coupled to form a branched peptide.

16. The indolicidin analogue according to claim 15, wherein four analogues are coupled to a peptide core having the formula: ##STR2##

17. The indolicidin analogue according to claim 15, wherein eight analogues are coupled to a peptide core having the formula: ##STR3##

18. The indolicidin analogue according to any one of claims 1 to 15, wherein the analogue has one or more amino acids altered to a corresponding D-amino acid.

19. The indolicidin analogue according to claim 18, wherein the N-terminal amino acid is a D-amino acid.

20. The indolicidin analogue according to claim 18, wherein the C-terminal amino acid is a D-amino acid.

21. The indolicidin analogue according to claim 18, wherein the N-terminal amino acid and the C-terminal amino acid are D-amino acids.

22. The indolicidin analogue according to any one of claims 1-15, wherein the analogue is acetylated at the N-terminal amino acid.

23. The indolicidin analogue according to any one of claims 1-15, wherein the analogue is amidated at the C-terminal amino acid.

24. The indolicidin analogue according to any one of claims 1-15, wherein the analogue is esterified at the C-terminal amino acid.

25. The indolicidin analogue according to any one of claims 1-15, wherein the analogue is modified by incorporation of homoserine/homoserine lactone at the C-terminal amino acid.

26. The indolicidin analogue according to any one of claims 1-15, wherein the analogue is conjugated with polyethylene glycol or derivatives thereof.

27. An isolated nucleic acid molecule whose sequence comprises one or

more coding sequences of an indolicidin analogue according to any one of claims 11-14.

28. An expression vector comprising a promoter in operable linkage with the nucleic acid molecule of claim 27.

29. A host cell transfected or transformed with the expression vector of claim 28.

30. A pharmaceutical composition comprising at least one indolicidin analogue according to any of claims 1-26 and a physiologically acceptable buffer.

31. The pharmaceutical composition according to claim 30, further comprising an antibiotic agent.

32. The pharmaceutical composition according to claim 31, wherein the antibiotic is selected from the group consisting of penicillins, cephalosporins, carbacephems, cephamycins, carbapenems, monobactams, quinolones, tetracyclines, aminoglycosides, macrolides, glycopeptides, chloramphenicols, glycylcyclines, licosamides and fluoroquinolones.

33. The pharmaceutical composition according to claim 31, wherein the antibiotic is selected from the group consisting of Amikacin; Amoxicillin; Ampicillin; Azithromycin; Azlocillin; Aztreonam; Carbenicillin; Cefaclor; Cefamandole formate sodium; Cefazolin; Cefepime; Cefetamet; Cefixime; Cefmetazole; Cefonicid; Cefoperazone; Cefotaxime; Cefotetan; Cefoxitin; Cefpodoxime; Cefprozil; Cefsulodin; Ceftazidime; Ceftizoxime; Ceftriaxone; Cefuroxime; Cephalexin; Cephalothin; Chloramphenicol; Cinoxacin; Ciprofloxacin; Clarithromycin; Clindamycin; Cloxacillin; Co-amoxiclavulanate; Dicloxacillin; Doxycycline; Enoxacin; Erythromycin; Erythromycin estolate; Erythromycin ethyl succinate; Erythromycin glucoheptonate; Erythromycin lactobionate; Erythromycin stearate; Ethambutol; Fleroxacin; Gentamicin; Imipenem; Isoniazid; Kanamycin; Lomefloxacin; Loracarbef; Meropenem Methicillin; Metronidazole; Mezlocillin; Minocycline hydrochloride; Mupirocin; Nafcillin; Nalidixic acid; Netilmicin; Nitrofurantoin; Norfloxacin; Ofloxacin; Oxacillin; Penicillin G; Piperacillin; Pyrazinamide; Rifabutin; Rifampicin; Roxithromycin; Streptomycin; Sulfamethoxazole; Synercid; Teicoplanin; Tetracycline; Ticarcillin; Tobramycin; Trimethoprim; Vancomycin; a combination of Piperacillin and Tazobactam; and derivatives thereof.

34. The pharmaceutical composition according to claim 31, wherein the antibiotic is selected from the group consisting of Amikacin; Amoxicillin; Ampicillin; Azithromycin; Cefoxitin; Ceftriaxone; Ciprofloxacin; Clarithromycin; Doxycycline; Erythromycin; Gentamicin; Mupirocin; Piperacillin; Teicoplanin; Tobramycin; Vancomycin; and a combination of Piperacillin and Tazobactam.

35. A pharmaceutical composition comprising a physiologically acceptable buffer and a combination of an analogue and an antibiotic, wherein the combination is selected from the group consisting of:

Ile Leu Lys Lys Phe Pro Phe Phe Pro Phe Arg Arg Lys and ciprofloxacin

Ile Leu Lys Lys Phe Pro Phe Phe Pro Phe Arg Arg Lys and vancomycin.

Ile Leu Arg Arg Trp Pro Trp Trp Pro Trp Arg Arg Arg and piperacillin.

Ile Leu Arg Trp Pro Trp Trp Pro Trp Arg Arg Lys Ile Met Ile Leu Lys Lys Ala Gly Ser and gentamicin,

Trp Arg Ile Trp Lys Pro Lys Trp Arg Leu Pro Lys Trp and vanconlycin.

Trp Arg Ile Trp Lys Pro Lys Trp Arg Leu Pro Lys Trp and tobrarnycin.

Trp Arg Ile Trp Lys Pro Lys Trp Arg Leu Pro Lys Trp and piperacillin.

Ile Leu Lys Lys Trp Pro Trp Trp Pro Trp Arg Arg Lys and piperacillin.

Ile Leu Lys Lys Trp Val Trp Trp Pro Trp Arg Arg Lys and tobraniycin and

Ile Leu Arg Trp Val Trp Trp Val Trp Arg Arg Lys and piperaclilin.

36. The pharmaceutical composition according to claim 30, further comprising an antiviral agent.

37. The pharmaceutical composition according to claim 36 wherein the antiviral agent is selected from the group consisting of acyclovir; amantadine hydrochloride; didanosine; edoxudine; famciclovir; foscarnet; ganciclovir; idoxuridine; interferon; lamivudine; nevirapine; penciclovir; podophyllotoxin; ribavirin; rimantadine; sorivudine; stavudine; trifluridine; vidarabine; zalcitabine and zidovudine.

38. The pharmaceutical composition according to claim 30, further comprising an antiparasitic agent.

39. The pharmaceutical composition according to claim 38 wherein the antiparasitic agent is selected from the group consisting of 8-hydroxyquinoline derivatives; cinchona alkaloids; nitroimidazole derivatives; piperazine derivatives; pyrimidine derivatives and quinoline derivatives.

40. The pharmaceutical composition according to claim 38 wherein the antiparasitic agent is selected from the group consisting of albendazole; atovaquone; chloroquine phosphate; diethylcarbamazine citrate; eflomithine; halofantrine; iodoquinol; ivermectin; mebendazole; mefloquine hydrochloride; melarsoprol B; metronidazole; niclosamide; nifurtimox; paromomycin; pentamidine isethionate; piperazine; praziquantel; primaquine phosphate; proguanil; pyrantel pamoate; pyrimethamine; pyrvinium pamoate; quinidine gluconate; quinine sulfate; sodium stibogluconate; suramin and thiabendazole.

41. The pharmaceutical composition according to claim 30, further comprising an antifungal agent.

42. The pharmaceutical composition according to claim 41, wherein the antifungal agent is selected from the group consisting of allylamines; imidazoles; pyrimidines and triazoles.

43. The pharmaceutical composition according to claim 41, wherein the antifungal agent is selected from the group consisting of 5-fluorocytosine; amphotericin B; butoconazole; chlorphenesin; ciclopirox; clioquinol; clotrimazole; econazole; fluconazole; flucytosine; griseofulvin; itraconazole; ketoconazole; miconazole; naftifine hydrochloride; nystatin; selenium sulfide; sulconazole; terbinafine hydrochloride; terconazole; tioconazole; tolnaftate and undecylenate.

44. The pharmaceutical composition according to claim 30, wherein the composition is incorporated in a liposome.

45. The pharmaceutical composition according to claim 30, wherein the composition is incorporated in a slow-release vehicle.

46. A method of treating an infection, comprising administering to a patient a therapeutically effective amount of a pharmaceutical composition according to any of claims 30-45.

47. The method of claim 46, wherein the infection is due to a microorganism.

48. The method of claim 47, wherein the microorganism is selected from the group consisting of bacterium, fungus, parasite and virus.

49. The method of claim 48, wherein the fungus is a yeast and/or mold.

50. The method of claim 48, wherein the parasite is selected from the group consisting of protozoan, nematode, cestode and trematode.

51. The method of claim 50, wherein the parasite is a protozoan and is selected from the group consisting of Babesia spp.; Balantidium coli; Blastocystis hominis; Cryptosporidium parvum; Encephalitozoon spp.; Entamoeba spp.; Giardia lamblia; Leishmania spp.; Plasmodium spp.; Toxoplasma gondii; Trichomonas spp. and Trypanosoma spp.

52. The method of claim 50, wherein the parasite is selected from the group consisting of Ascaris lumbricoides; Clonorchis sinensis; Echinococcus spp.; Fasciola hepatica; Fasciolopsis buski; Heterophyes heterophyes; Hymenolepis spp.; Schistosoma spp.; Taenia spp. and Trichinella spiralis.

53. The method of claim 48, wherein the bacterium is a Gram-negative bacterium.

54. The method of claim 53, wherein the Gram-negative bacterium is selected from the group consisting of Acinetobacter spp.; Enterobacter spp.; E. coli; H. influenzae;, K. pneumoniae; P. aeruginosa; S. marcescens and S. maltophilia.

56. The method of claim 53, wherein the Gram-negative bacterium is selected from the group consisting of Bordetella pertussis; Brucella spp.; Campylobacter spp.; Haemophilus ducreyi; Helicobacter pylori; Legionella spp.; Moraxella catarrhalis; Neisseria spp.; Salmonella spp.; Shigella spp. and Yersinia spp.

57. The method of claim 48, wherein the bacterium is a Gram-positive bacterium.

58. The method of claim 57, wherein the Gram-positive bacterium is selected from the group consisting of E. faecalis; S. aureus; E. faecium; S. pyogenes; S. pneumoniae and coagulase-negative staphylococci.

59. The method of claim 57, wherein the Gram-positive bacterium is selected from the group consisting of Bacillus spp.; Corynebacterium spp.; Diphtheroids; Listeria spp. and Viridans Streptococci.

60. The method of claim 48, wherein the bacterium is an anaerobe.

61. The method of claim 60, wherein the anaerobe is selected from the group consisting Clostridium spp., Bacteroides spp. and Peptostreptococcus spp.

62. The method of claim 48, wherein the bacterium is selected from the group consisting of Borrelia spp.; Chlamydia spp.; Mycobacterium spp.; Mycoplasma spp.; Propionibacterium acne; Rickettsia spp.; Treponema spp. and Ureaplasma spp.

63. The method of claim 48, wherein the virus is an RNA virus selected from the group consisting of Alphavirus; Arenavirus; Bunyavirus; Coronavirus; Enterovirus; Filovirus; **Flavivirus**; Hantavirus; HTLV-BLV; Influenzavirus; Lentivirus; Lyssavirus; Paramyxovirus; Reovirus; Rhinovirus and Rotavirus.

64. The method of claim 48, wherein the virus is a DNA virus selected from the group consisting of Adenovirus; **Cytomegalovirus**;

hepadnavirus; Molluscipoxvirus; Orthopoxvirus; Papillomavirus; Parvovirus; Polyomavirus; Simplexvirus and Varicellovirus.

65. The method of claim 46, wherein the pharmaceutical composition is administered by intravenous injection, intraperitoneal injection or implantation, intramuscular injection or implantation, intrathecal injection, subcutaneous injection or implantation, intradermal injection, lavage, bladder wash-out, suppositories, pessaries, oral ingestion, topical application, enteric application, inhalation, aerosolization or nasal spray or drops.

66. A composition, comprising an indolicidin analogue according to any of claims 1-26 and an antibiotic.

67. A device coated with a composition comprising the indolicidin analogue according to claims 1-26.

68. The device of claim 67, wherein the composition further comprises an antibiotic agent.

69. The device of either of claims 67 or 68, wherein the device is a medical device.

70. An antibody that reacts specifically with the analogue according to any of claims 11-14.

71. The antibody of claim 70, wherein the antibody is a monoclonal antibody or single chain antibody.

72. A composition comprising a compound modified by derivatization of an amino group with a conjugate comprising activated polyoxyalkylene glycol and a fatty acid.

73. The composition of claim 72, wherein the conjugate further comprises sorbitan linking the polyoxyalkylene glycol and fatty acid.

74. The composition of claim 72, wherein the conjugate is polysorbate.

75. The composition of claim 72, wherein the fatty acid has from 12 to 18 carbons.

76. The composition of claim 72, wherein the polyoxyalkylene glycol is polyoxyethylene.

77. The composition of claim 76, wherein the polyoxyethylene has a chain length of from 2 to 100 monomeric units.

78. The composition of claim 72, wherein the compound is a peptide or protein.

79. The composition of claim 72, wherein the compound is a cationic peptide.

80. The composition of claim 79, wherein the cationic peptide is indolicidin, an indolicidin analogue or a cecropin/melittin fusion peptide.

81. The composition of claim 72, wherein the polyoxyalkylene glycol is activated by irradiation with ultraviolet light.

82. A method of overcoming tolerance of a bacterium to an antibacterial agent, comprising: contacting the bacterium with a composition comprising the antibacterial agent and a cationic peptide, therefrom overcoming tolerance.

83. The method of claim 82, wherein the cationic peptide is selected

from the group consisting of Abaecins, Andropins, Apidaecins, AS, Bactenecins, Bac, Bactericidins, Bacteriocins, Bombinins, Bombolitins, BPTI, Brevinins, CAP 18 and related peptides, Cecropins, Ceratotoxins, Charybdtoxins, Coleoptericins, Crabolins, alpha, beta, and insect defensins, Dermaseptins, Diptericins, Drosocins, Esculentins, Gramicidins, Histatins, Indolicidins, Lactoferricins, Lantibiotics, Leukocins, Magainins and related peptides from amphibians, Mastoparans, Melittins, Phormicins, Polyphemusins, Protegrins, Royalisins, Sarcotoxins, Seminal plasmins, Sepacins, Tachyplesins, Thionins, Toxins, Cecropin-Melittin chimeras and analogues thereof.

84. The method of claim 82, wherein the cationic peptide is an indolicidin analogue.

85. A method of overcoming inherent resistance of a microorganism to an antibiotic agent, comprising: contacting the microorganism to a composition comprising the antibiotic agent and a cationic peptide selected from the group consisting of Abaecins, Andropins, Apidaecins, AS, Bactenecins, Bac, Bactericidins, Bacteriocins, Bombinins, Bombolitins, Brevinins, CAP 18 and related peptides, Cecropins, Ceratotoxins, Charybdtoxins, Coleoptericins, Crabolins, Dermaseptins, Diptericins, Drosocins, Esculentins, Gramicidins, Histatins, Indolicidins, Lactoferricins, Lantibiotics, Leukocins, Magainins and related peptides from amphibians, , Mastoparans, Melittins, Phormicins, Polyphemusins, Protegrins, Royalisins, Sarcotoxins, Seminal plasmins, Sepacins, Tachyplesins, Thionins, Toxins, Cecropin-Melittin chimeras and analogues thereof, therefrom overcoming inherent resistance.

86. The method of claim 85, wherein the cationic peptide is an indolicidin analogue.

87. A method of overcoming acquired resistance of a microorganism to an antibiotic agent, comprising: contacting the microorganism to a composition comprising the antibiotic agent and a cationic peptide selected from the group consisting of Abaecins, Andropins, Apidaecins, AS, Bactenecins, Bac, Bactericidins, Bacteriocins, Bombinins, Bombolitins, Brevinins, CAP 18 and related peptides, Cecropins, Ceratotoxins, Charybdtoxins, Coleoptericins, Crabolins, alpha, beta, and insect Defensins, Dermaseptins, Diptericins, Drosocins, Esculentins, Gramicidins, Histatins, Indolicidins, Lactoferricins, Lantibiotics, Leukocins, Magainins and related peptides from amphibians, Mastoparans, Melittins, Phormicins, Protegrins, Royalisins, Sarcotoxins, Seminal plasmins, Sepacins, Thionins, Toxins, Cecropin-Melittin chimeras and analogues thereof, therefrom overcoming acquired resistance.

88. The method of claim 87, wherein the cationic peptide is an indolicidin analogue.

89. A method of overcoming tolerance of a bacterium to an antibacterial agent, overcoming inherent resistance of a microorganism an antibacterial agent, overcoming acquired resistance of a microorganism an antibacterial agent or enhancing the activity of an antibiotic agent against a susceptible microorganism, comprising administering a pharmaceutical composition of lysozyme or nisin and an antibacterial agent, therefrom overcoming tolerance, inherent resistance, acquired reistance, or enhancing activity.

90. A method of enhancing activity of an antibiotic agent against a susceptible microorganism, comprising administering a pharmaceutical composition comprising the antibiotic agent and a cationic peptide selected from the group consisting of Abaecins, Andropins, Apidaecins, AS, Bactenecins, Bac, Bactericidins, Bacteriocins, Bombinins, Bombolitins, Brevinins, CAP 18 and related peptides, Ceratotoxins, Charybdtoxins, Coleoptericins, alpha, beta, and insect Defensins, Dermaseptins, Diptericins, Drosocins, Esculentins, Gramicidins, Histatins, Indolicidins, Leukocins, Mastoparans, Phormicins,

Polyphemusins, Protegrins, Royalisins, Seminal plasmins, Sepacins, Thionins, Toxins and analogues thereof, therefrom enhancing activity of the antibiotic agent against the susceptible microorganism.

91. The method of claim 90, wherein the cationic peptide is an indolicidin analogue.

92. The method of claim 89, wherein the cationic peptide and antibacterial agents are selected from the group consisting of:

MBI 11A1CN	Chloramphenicol;
MBI 11B4CN	Erythromycin;
MBI 21A10	Ampicillin;
MBI 21A10	Piperacillin;
MBI 26	Vancomycin;
MBI 29	Gentamicin and
MBI 29A3	Penicillin.

93. The method of claim 85, wherein the cationic peptide and antibiotic agents are selected from the group consisting of:

MBI 11B16CN	Amikacin;
MBI 11D18CN	Gentamicin;
MBI 11D18CN	Gentamicin;
MBI 21A1	Mupirocin;
MBI 21A1	Tobramycin;
MBI 26	Amikacin;
MBI 26	Gentamicin;
MBI 29A3	Amikacin;
MBI 29A3	Tobramycin and
MBI 29F1	Amikacin.

94. The method of claim 87, wherein the cationic peptide and antibiotic agents are selected from the group consisting of:

MBI 11A1CN	Vancomycin;
MBI 11B16CN	Gentamicin;
MBI 11D18CN	Gentamicin;
MBI 11F3CN	Tobramycin;
MBI 11F4CN	Piperacillin;
MBI 21A1	Tobramycin;
MBI 26	Ceftriaxone;
MBI 26	Vancomycin;
MBI 29A2	Ciprofloxacin and
MBI 29A2	Ciprofloxacin.

95. The method of claim 90, wherein the cationic peptide and antibiotic agents are selected from the group consisting of:

MBL 11B16CN	Amikacin;
MBI 11CN	Piperacillin;
MBI 11G13CN	Tobramycin;
MBI 11G7CN	Piperacillin;
MBI 11J02CN	Ceftriaxone;
MBI 21A2	Gentamicin;
MBI 28	Mupirocin;
MBI 29	Vancomycin;
MBI 29A2	Ciprofloxacin and
REWH 53A5CN	Tobramycin.

2001:107071 DNA vaccines against tick borne **flaviviruses**.
Schmaljohn, Connie S., Frederick, MD, United States
The United States of America as represented by the Secretary of the Army, Washington, DC, United States (U.S. corporation)
US 6258788 B1 20010710
APPLICATION: US 1998-197218 19981120 (9) <--
PRIORITY: US 1997-65750P 19971120 (60)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Particle mediated immunization of tick-borne **flavivirus** genes confers homologous and heterologous protection against tick borne encephalitis.
CLM What is claimed is:
1. A method for inducing a protective immune response to a tick-borne **flavivirus** protein in a mammal, comprising (i) preparing a nucleic acid encoding an antigenic determinant of a tick-borne **flavivirus** prM/E protein operatively linked to a **CMV** promoter operative in cells of a mammal, which nucleic acid is suitable for stably producing the antigenic determinant in a mammal; (ii) coating the nucleic acid in (i) onto carrier particles; (iii) accelerating the coated carrier particles into epidermal cells of the mammal in vivo; and (iv) inducing a protective immune response in said mammal upon exposure to a tick-borne **flavivirus**.

2. The method according to claim 1 wherein the carrier particles are gold.

3. The method according to claim 1 wherein the tick-borne **flavivirus** prM/E protein is selected from the group consisting of Russian spring summer encephalitis prM/E proteins, and Central European encephalitis prM/E proteins.

4. The method according to claim 1 wherein the nucleic acid encodes a protein coding region comprising SEQ ID NO:1.

5. A method for inducing a protective immune response to a tick-borne **flavivirus** protein in a mammal, comprising (i) preparing a nucleic acid encoding an antigenic determinant of a Russian spring summer encephalitis tick-borne **flavivirus** prM/E protein operatively linked to a promoter operative in cells of a mammal, which nucleic acid encodes a protein coding region comprising SEQ ID NO:2 and is suitable for stably producing the antigenic determinant in a mammal; (ii) coating the nucleic acid in (i) onto carrier particles; (iii) accelerating the coated carrier particles into epidermal cells of the mammal in vivo; and (iv) inducing a protective immune response in said mammal upon exposure to a tick-borne **flavivirus**.

6. The method according to claim 1 wherein the nucleic acid encodes a protein coding region comprising SEQ ID NO: 1 and SEQ ID NO:2.

7. A kit for inducing a protective immune response to a tick-borne **flavivirus** protein in a mammal, comprising packaged in association: (a) a nucleic acid encoding an antigenic determinant of a tick-borne **flavivirus** prM/E protein operatively linked to a **CMV** promoter operative in cells of a mammal, which nucleic acid is suitable for stably producing the antigenic determinant in a mammal; (b) one or both of a coating solution and/or components of a coating solution; and (c) carrier particles.

8. The kit of claim 7, wherein the tick-borne **flavivirus** prM/E protein is selected from the group consisting of Russian spring summer encephalitis prM/E proteins, and Central European encephalitis prM/E proteins.

9. The kit of claim 7, wherein the nucleic acid encodes a protein coding region comprising SEQ ID NO:1.

10. The kit of claim 7, wherein the nucleic acid encodes a protein coding region comprising SEQ ID NO:1 and SEQ ID NO:2.

11. A kit for inducing a protective immune response to a tick-borne **flavivirus** protein in a mammal, comprising packaged in association: (a) a nucleic acid encoding an antigenic determinant of a Russian spring summer encephalitis tick-borne **flavivirus** prM/E protein operatively linked to a promoter operative in cells of a mammal which nucleic acid encodes a protein coding region comprising SEQ ID NO:2 and is suitable for stably producing the antigenic determinant in a mammal; (b) one or both of a coating solution and/or components of a coating solution; and (c) carrier particles.

L23 ANSWER 3 OF 10 USPATFULL on STN
2001:63426 Dendritic-marker expressing macrophage cultures and methods of reactivating latent virus.
Soderberg-Naucler, Cecilia E., Hagarsten, Sweden
Fish, Kenneth N., San Diego, CA, United States
Moses, Ashlee, Portland, OR, United States
Streblow, Daniel, Tigard, OR, United States
Nelson, Jay, Tualatin, OR, United States
Oregon Health Sciences University, Portland, OR, United States (U.S. corporation)
US 6225048 B1 20010501
APPLICATION: US 1998-164221 19980930 (9) <--
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention provides methods of latent virus reactivation in monocyte-derived macrophages through allogeneic stimulation of peripheral blood mononuclear cells ("PBMC"), methods of culturing virus, and cultures of virally permissive monocyte-derived macrophages.
CLM What is claimed is:
1. A method of replicating viruses in virally permissive monocyte-derived macrophages, the method comprising the steps of: (a) culturing monocytes under conditions where the monocytes are in fluid communication with viable, allogeneically stimulated peripheral blood mononuclear cells (PBMC) to activate the monocytes to differentiate into virally permissive monocyte-derived macrophages; and (b) permitting viral replication in the monocyte-derived macrophages, where the virus is either latent in the monocytes or exogenously added to the culture, and wherein the monocyte derived-macrophages have a majority population of cells bearing CD83 and CD14.

2. A method of claim 1, wherein the monocyte derived-macrophages have a majority population of cells bearing CD83, CD68, CD1a, CD64, and CD14.

3. A method of claim 1, wherein the allogeneically stimulated cells include CD4+ and CD8+ cells.

4. A method of claim 1, wherein the virus is selected from the group consisting of **cytomegalovirus** (**CMV**), hepatitis C virus (HCV), human immunodeficiency virus (HIV), human herpes virus 6 (HHV6), human herpes virus 7 (HHV7), and human herpes virus 8 (HHV8).

5. A method of claim 4, wherein the virus is **CMV**.

6. A method of claim 1, wherein the monocyte-derived macrophages are human.

7. A method for screening for inhibitors of virus production using virally permissive monocyte-derived macrophages, the method comprising the steps of: (a) culturing monocytes under conditions where the monocytes are in fluid communication with viable, allogeneically stimulated peripheral blood mononuclear cells (PBMC) to activate the monocytes to differentiate into virally permissive monocyte-derived

macrophages, (b) permitting viral replication in the monocyte derived macrophages, where the virus is either latent in the monocytes or exogenously added to the culture; (c) contacting the monocyte-derived macrophages of step (b) with substances suspected of having the ability to inhibit viral production; and (d) detecting the level of virus production in the monocytes-derived macrophages.

8. A method of claim 7, wherein the monocyte-derived macrophages have a majority population of cell bearing CD83 and CD14.

9. A method of claim 7, wherein the monocyte derived-macrophages have a majority population of cells bearing CD83, CD68, CD1a, CD64, and CD14.

10. A method of claim 7, wherein the allogeneically stimulated cells include CD4+ and CD8+ cells.

11. A method of claim 7, wherein the virus is latent.

12. A method of claim 7, wherein the monocyte-derived macrophages are human.

13. A method of claim 7, wherein the substances are inhibitors of viral proteases.

14. A method of claim 7, wherein the substances are antisense molecules that bind to nucleic acid generated by the virus.

15. A method of claim 7, wherein the substances are antisense molecules that are complementary to mRNA encoded by a viral genome.

16. A method of claim 15, wherein the substances are ribozymes complementary to MRNA encoded by a viral genome.

17. A method of claim 7, wherein the virus is selected from the group consisting of **cytomegalovirus** (**CMV**), hepatitis C virus (HCV), human immunodeficiency virus (HIV), human herpes virus 6 (HHV6), human herpes virus 7 (HHV7), and human herpes virus 8 (HHV8).

18. A method of claim 17, wherein the virus is **CMV**.

19. A method of claim 17, wherein the substances inhibit a viral protein selected from the group consisting of **CMV** DNA polymerase, UL80, and UL89.

20. A stable culture of virally permissive monocyte-derived macrophages, wherein the monocyte-derived macrophages are derived from monocytes exposed to allogeneically stimulated peripheral blood mononuclear cells (PBMC) for a time sufficient to and a concentration sufficient to: (i) stimulate active differentiation of the monocytes into monocyte-derived macrophages, and (ii) stimulate viral production in the monocyte-derived macrophages; and, wherein the virally permissive monocyte-derived macrophages produce at least 10,000 fold greater virus than non-allogeneically stimulated monocytes, and wherein the monoczte derived-macrophages have a majority population of cells bearing CD83 and CD14.

21. A culture of claim 20, wherein the monocyte derived-macrophages have a majority population of cells bearing CD83, CD68, CD1a, CD64, and CD14.

22. A culture of claim 20, wherein the monocyte-derived macrophages produce a virus selected from the group consisting of **cytomegalovirus** (**CMV**), hepatitis C virus (HCV), human immunodeficiency virus (HIV), human herpes virus 6 (HHV6), human herpes virus 7 (HHV7), and human herpes virus 8 (HHV8).

23. A culture of claim 22, wherein the virus is **CMV**.

24. A stable culture of virally permissive monocyte-derived macrophages having a population defined as at least 85% bearing CD83 and CD14.

25. A culture of claim 24, wherein the monocyte derived-macrophages have a population of at least 85% of the cells bearing CD83, CD68, CD1a, CD64, and CD14.

26. A culture of claim 24, wherein the virus is latent.

27. A culture of claim 24, wherein the monocyte-derived macrophages produce a virus selected from the group consisting of **cytomegalovirus** (**CMV**), hepatitis C virus (HCV), human immunodeficiency virus (HIV), human herpes virus 6 (HHV6), human herpes virus 7 (HHV7), and human herpes virus 8 (HH8).

28. A culture of claim 27, wherein the virus is **CMV**.

29. A culture of claim 24, wherein the monocyte-derived macrophages are human.

30. A method of culturing virally permissive monocyte-derived macrophages, the method comprising the step of culturing monocytes under conditions where the monocytes are in fluid communication with viable, allogeneically stimulated peripheral blood mononuclear cells (PBMC) to activate the monocytes to differentiate into monocyte-derived macrophages, wherein the monocyte derived-macrophages have a majority population of cells bearing CD83 and CD14.

31. A method of claim 30, wherein the monocyte derived-macrophages have a majority population of cells bearing CD83, CD68, CD1a, CD64, and CD14.

32. A method of claim 30, wherein the allogeneically stimulated cells include CD4+ and CD8+ cells.

33. A method of claim 30, wherein the monocyte-derived macrophages are human.

34. A stable culture of virally permissive monocyte-derived macrophages having the following characteristics: (i) comprising dendritic cell markers CD68, CD83, and CD1a; (ii) comprising macrophage cell markers CD64 and CD14; and (iii) derived from CD14+ monocytes.

35. A method of replicating viruses in virally permissive monocyte-derived macrophages, the method comprising the steps of: (a) culturing CD14+ monocytes under conditions where the monocytes are exposed to conditioned media comprising IFNγ in an amount sufficient to activate the monocytes to differentiate into virally permissive monocyte-derived macrophages; and (b) permitting viral replication in the monocyte-derived macrophages, where the virus is either latent in the monocytes or exogenously added to the culture, and wherein the monocyte derived-macrophages have a majority population of cells bearing CD83 and CD14.

36. A method of claim 35, wherein the monocyte derived-macrophages have a majority population of cells bearing CD83, CD68, CD1a, CD64, and CD14.

37. A method of claim 35, wherein the virus is selected from the group consisting of **cytomegalovirus** (**CMV**), hepatitis C virus (HCV), human immunodeficiency virus (HIV), human herpes virus 6 (HHV6), human herpes virus 7 (HHV7), and human herpes virus 8 (HHV8).

38. A method of claim 37, wherein the virus is **CMV**.

39. A method of claim 35, wherein the monocyte-derived macrophages are human.

40. A method for screening for inhibitors of virus production using virally permissive monocyte-derived macrophages, the method comprising the steps of: (a) culturing CD 14+ monocytes under conditions where the monocytes exposed to conditioned media comprising IFNγ in an amount sufficient to activate the monocytes to differentiate into virally permissive monocyte-derived macrophages; (b) permitting viral replication in the monocyte-derived macrophages, where the virus is either latent in the monocytes or exogenously added to the culture; (c) contacting the monocyte-derived macrophages of step (b) with substances suspected of having the ability to inhibit viral production; and (d) detecting the level of virus production in the monocytes-derived macrophages.

41. A method of claim 40, wherein the monocyte-derived macrophages have a majority population of cell bearing CD83 and CD14.

42. A method of claim 40, wherein the monocyte derived-macrophages have a majority population of cells bearing CD83, CD68, CD1a, CD64, and CD14.

43. A method of claim 40, wherein the virus is latent.

44. A method of claim 40, wherein the monocyte-derived macrophages are human.

45. A method of claim 40, wherein the virus is selected from the group consisting of **cytomegalovirus** (**CMV**), hepatitis C virus (HCV), human immunodeficiency virus (HIV), human herpes virus 6 (HHV6), human herpes virus 7 (HHV7), and human herpes virus 8 (HHV8).

46. A method of claim 45, wherein the virus is **CMV**.

47. A stable culture of virally permissive monocyte-derived macrophages, wherein the monocyte-derived macrophages are derived from CD14+ monocytes exposed to conditioned media comprising IFNγ in an amount sufficient to and for a time sufficient to: (i) stimulate active differentiation of the CD14+ monocytes into monocyte-derived macrophages, and (ii) stimulate viral production in the monocyte-derived macrophages; and, wherein the virally permissive monocyte-derived macrophages produce at least 10,000 fold greater virus than non-allogeneically stimulated monocytes.

48. A culture of claim 47, wherein the majority of the monocyte-derived macrophages bear CD83 and CD14.

49. A culture of claim 47, wherein the majority of the monocyte-derived macrophages bear CD83, CD68, CD1a, CD64, and CD14.

50. A culture of claim 47, wherein the monocyte-derived macrophages produce a virus selected from the group consisting of **cytomegalovirus** (**CMV**), hepatitis C virus (HCV), human immunodeficiency virus (HIV), human herpes virus 6 (HHV6), human herpes virus 7 (HHV7), and human herpes virus 8 (HHV8).

51. A culture of claim 50, wherein the virus is **CMV**.

52. A stable culture of virally permissive monocyte-derived macrophages having a population defined as at least 85% bearing CD83 and CD14.

53. A culture of claim 52, wherein the population has at least 85% of the cells bearing CD83, CD68, CD1a, CD64, and CD14.

54. A culture of claim 52, wherein the virus is latent.

55. A culture of claim 52, wherein the monocyte-derived macrophages produce a virus selected from the group consisting of **cytomegalovirus**

(CMV), hepatitis C virus (HCV), human immunodeficiency virus (HIV), human herpes virus 6 (HHV6), human herpes virus 7 (HHV7), and human herpes virus 8 (HHV8).

56. A culture of claim 55, wherein the virus is **CMV**.

57. A culture of claim 52, wherein the monocyte-derived macrophages are human.

58. A method of culturing virally permissive monocyte-derived macrophages, the method comprising the step of culturing CD14+ monocytes under conditions where the monocytes are exposed to conditioned media comprising IFNγ in an amount sufficient to activate the monocytes to differentiate into virally permissive monocyte-derived macrophages, and wherein the monocyte derived-macrophages have a majority population of cells bearing CD83 and CD14.

59. A method of claim 58, wherein the monocyte derived-macrophages have a majority population of cells bearing CD83, CD68, CD1a, CD64, and CD14.

60. A method of claim 58, wherein the monocyte-derived macrophages are human.

61. The method of claim 1, wherein the virus is hepatitis C virus (HCV).

62. The method of claim 7, wherein the virus is hepatitis C virus (HCV).

63. The method of claim 20, wherein the virus is hepatitis C virus (HCV).

64. The method of claim 35, wherein the virus is hepatitis C virus (HCV).

65. The method of claim 40, wherein the virus is hepatitis C virus (HCV).

66. The method of claim 47, wherein the virus is hepatitis C virus (HCV).

67. Virus made using the method of claim 1.

68. The virus of claim 67, wherein the virus is selected from the group consisting of **cytomegalovirus** (**CMV**), hepatitis C virus (HCV), human immunodeficiency virus (HIV), human herpes virus 6 (HHV6), human herpes virus 7 (HHV7), and human herpes virus 8 (HHV8).

69. Virus of claim 67, wherein, the virus is hepatitis C virus (HCV).

70. Infective virus made using the method of claim 38.

71. The virus of claim 70, wherein the virus is selected from the group consisting of **cytomegalovirus** (**CMV**), hepatitis C virus (HCV), human immunodeficiency virus (HIV), human herpes virus 6 (HHV6), human herpes virus 7 (HHV7), and human herpes virus 8 (HHV8).

72. The virus of claim 70, wherein the virus is hepatitis C virus (HCV).

73. A method of infecting cells with virus, the method comprising the steps of: (a) culturing monocytes under conditions where the monocytes are in fluid communication with viable, allogeneically stimulated peripheral blood mononuclear cells (PBMC) to activate the monocytes to differentiate into virally permissive monocyte-derived macrophages; (b) permitting virus replication in the monocyte-derived macrophages, where the virus is either latent in the monocytes or exogenously added to the culture; (c) collecting virus from the culture; and (d) contacting a

second culture of cells with the virus, thereby infecting the cells with the virus.

74. The method of claim 73, wherein the second culture of cells comprises fibroblasts.

75. The method of claim 73, wherein the virus is selected from the group consisting of **cytomegalovirus** (**CMV**), hepatitis C virus (HCV), human immunodeficiency virus (HIV), human herpes virus 6 (HHV6), human herpes virus 7 (HHV7), and human herpes virus 8 (HHV8).

76. The method of claim 73, wherein the virus is hepatitis C virus (HCV).

77. A method of infecting cells with virus, the method comprising the steps of: (a) culturing CD14+ monocytes under conditions where the monocytes are exposed to conditioned media comprising IFNγ in an amount sufficient to activate the monocytes to differentiate into virally permissive monocyte-derived macrophages; (b) permitting viral replication in the monocyte-derived macrophages, where the virus is either latent in the monocytes or exogenously added to the culture. (c) collecting virus from the culture; and (d) contacting a second culture of cells with the virus, thereby infecting the cells with the virus.

78. The method of claim 77, wherein the second culture of cells comprises fibroblasts.

79. The method of claim 77, wherein the virus is selected from the group consisting of **cytomegalovirus** (**CMV**), hepatitis C virus (HCV), human immunodeficiency virus (HIV), human herpes virus 6 (HHV6), human herpes virus 7 (HHV7), and human herpes virus 8 (HHV8).

80. The method of claim 77, wherein the virus is hepatitis C virus (HCV).

L23 ANSWER 4 OF 10 USPATFULL on STN
2001:44013 Lentiviral vectors.
Chang, Lung-Ji, 3102 NW. 57th Terr., Gainesville, FL, United States 32606-6939
US 6207455 B1 20010327
APPLICATION: US 1997-935312 19970922 (8) <--
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention contemplates novel lentiviral vectors which exhibit strong promoter activity in human and other cells. Vectors are provided which are packaged efficiently in packaging cells and cell lines to generate high titer recombinant virus stocks. The present invention further relates to HIV vaccines and compositions for gene therapy. In particular, the present invention provides attenuated replication-competent HIV vaccines and replication-defective HIV vectors.
CLM What is claimed is:
1. A packaging vector comprising a nucleotide sequence encoding Gag and Pol proteins of a reference lentivirus, said packaging vector differing from said reference lentivirus at least in that (a) it lacks a functional major splice donor site, or its manor splice donor site, while functional, differs in sequence from that of said reference lentivirus, and (b) it lacks a functional major packaging signal, which vector, after introduction into a suitable host cell, is capable of causing such cell, either through expression from said vector alone, or through co-expression from said vector and a second vector providing for expression of a compatible envelope protein, to produce packaging vector particles comprising functional Gag and Pol proteins and having a normal or a pseudotyped envelope, where said particles are free of the RNA form of said packaging vector as a result of (b) above, where said cell, as a

result of said expression of co expression, produces particles encapsulating the RNA form of a transducing vector possessing a compatible and functional packaging signal if said transducing vector is introduced into said cell, where said reference lentivirus is a human or simian immunodeficiency virus.

2. The packaging vector of claim 1 in which the reference lentivirus is HIV-1.

3. The packaging vector of claim 1 in which the reference lentivirus is HIV-2.

4. The packaging vector of claim 1 in which the reference lentivirus is SIV.

5. The packaging vector of claim 1 which encodes one or more envelope proteins.

6. The packaging vector of claim 1 which does not encode a functional envelope protein.

7. The packaging vector of claim 1 wherein the major splice donor site of said vector differs in sequence from that of any lentivirus major splice donor site sufficiently so that said major splice donor site is not a potential site for homologous recombination between said packaging vector and any HIV or SIV.

8. The packaging vector of claim 1 which comprises a sequence encoding a lentivirus Env proteins.

9. The packaging vector of claim 1 which comprises a sequence encoding the VSV-G envelope protein.

10. The packaging vector of claim 1 which further differs from said reference lentivirus in that at least portions of at least one gene selected from the group consisting of the env, vpr, vif, and vpu genes of said reference lentivirus is or are deleted.

11. The packaging vector of claim 1 which lacks the native primer binding site of said reference lentivirus.

12. The packaging vector of claim 1 which lacks the native polypurine tract of said reference lentivirus.

13. The packaging vector of claim 1 which lacks a functional nef gene.

14. The packaging vector of claim 1 which further differs from said lentivirus in that the 5' LTR has been modified.

15. The packaging vector of claim 1 in which the 5'LTR is a chimera of a lentivirus LTR and a **CMV** enhancer/promoter.

16. The packaging vector of claim 1 comprises a tat gene and a TAR sequence.

17. The packaging vector of claim 1 which comprises a rev gene and an RRE element.

18. The packaging vector of claim 1 which further differs from the reference lentivirus in that at least a portion of the tat gene and the TAR sequence are deleted.

19. The packaging vector of claim 1 which further differs from the reference lentivirus in that at least a portion of the env gene and the RRE element are deleted.

20. A packaging cell which comprises the packaging vector of claim 1 and is suitable for production of packaging or transducing vector particles.

21. A method of a producing a transducing vector comprising a remedial gene, in the form of an infectious particle, which comprises (a) transfecting a cell with a packaging vector according to claim 1, and, if said packaging vector does not itself provide for expression of a compatible envelope protein, a pseudotyping vector which does provide expression, so said cell is capable of producing packaging vector particles, (b) transfecting said cell with a transducing vector comprising said remedial gene, and a functional packaging signal, but which by itself is incapable of causing a cell to produce transducing vector particles, and (c) causing the cell to produce infectious transducing vector particles comprising said transducing vector in RNA form, said Gag and Pol proteins, and said envelope protein.

22. A kit comprising a packaging vector according to claim 1 and a transducing vector comprising a functional and compatible packaging signal, said transducing vector being incapable by itself of causing a cell transfected by said tranducing vector to encapsulate the RNA form of said transducing vector into a transducing vector particle.

23. The packaging vector of claim 1 in which the major splice donor site is a modified RSV major splice donor site corresponding to the splice donor site included in SEQ ID NO:9 and SEQ ID NO:10.

24. The packaging vector of claim 1 where said major splice donor site is functional but differs in sequence from that of all HIV and SIV lentivirus splice donor sites.

25. The packaging vector of claim 1 which lacks a functional major splice donor site.

26. The packaging vector of claim 1 where its major splice donor site, while functional, differs in sequence from that of said reference lentivirus sufficiently so that homologous recombination between said packaging vector and said reference lentivirus at said splice donor site is not detectable.

27. The vector of claim 1, wherein at least a portion of the env gene of said reference lentivirus is deleted.

28. The packaging vector of claim 7 wherein the major splice donor site of said vector is substantially identical to the RSV splice donor site.

29. The cell of claim 20, which further comprises a pseudotyping vector.

30. The cell of claim 20 which further comprises a transducing vector which by itself is incapable of coding for expression of infectious transducing vector particles, but which cell, as a result of the expression of genes of said packaging vector, packages the RNA form of said transducing vector into infectious transducing vector particles.

31. The cell of claim 20 where said transducing vector further comprises a remedial gene.

32. The cell of claim 20 wherein packaging is inducible.

33. The kit of claim 22, said packaging vector comprising a gene encoding a compatible envelope protein.

34. The kit of claim 22, further comprising a pseudotyping vector comprising a gene encoding a non-lentiviral envelope protein incorporatable into said particles.

35. The packaging vector of claim 26 in which the absence of detectable

homologous recombination is demonstrated by failure to detect replication-competent virus transfecting human TE671 cells with the packaging vector, co-culturing the TE671 cells with the human hymphoma cell line MT4 for two months, and determining, by immunohistochemical methods, whether the MT4 cells are producing HIV-1 proteins.

36. The vector of claim 27 in which the deletion is a frame shift mutation.

37. The vector of claim 27 in which two nucleotides of the env gene are deleted.

38. The vector of claim 27 in which 28 nucleotides of the env gene are deleted.

39. The vector of claim 27 in which the deletion is one achievable by Bal31 digestion at the unique NheI site in the env gene of wild-type HIV strain pNL4-3 or at the corresponding position in another reference lentivirus.

40. The vector of claim 35 where the presence of replication-competent virus is detected by determining by immunohistochemical methods whether the MT4 cells are producing HIV-1 proteins.

L23 ANSWER 5 OF 10 USPATFULL on STN
2000:18426 Chimeric hepatitis B/hepatitis C virus vaccine.
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Wakita, Takaji, Tokyo, Japan
The General Hospital Corporation, Charlestown, MA, United States (U.S. corporation)
US 6025341 20000215
APPLICATION: US 1997-854531 19970512 (8) <--
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Nucleic acid molecule that comprise an incomplete hepatitis C and hepatitis B viral genome including specifically disclosed DNA sequences are disclosed. Pharmaceutical compositions that contain nucleic acid molecules comprising an incomplete hepatitis C and hepatitis B viral genome including a nucleotide sequence encoding a complete hepatitis C core protein and hepatitis B S gene protein operably linked to regulatory elements functional in human cells are disclosed. Methods of immunizing individuals susceptible to or infected by hepatitis B virus and/or hepatitis C virus comprising the step of administering such pharmaceutical compositions are disclosed.
CLM What is claimed is:
1. A recombinant DNA molecule comprising a nucleotide coding sequence that encodes a fusion protein, wherein said fusion protein consists of a hepatitis B virus S gene product linked to amino acids 1-69 of the hepatitis C virus core protein.

2. The recombinant nucleic acid molecule of claim 1 wherein the N terminal amino acid of said hepatitis C virus core protein is linked to the C terminal amino acid of said hepatitis B virus S gene product.

3. A method of inducing an immune response against hepatitis C virus in an individual uninfected by hepatitis C virus comprising the step of: administering to said individual the DNA molecule of claim 1 in an amount effective to induce an immune response against hepatitis C virus.

4. A method of treating an individual who is infected with the hepatitis C virus comprising the step of: administering to said individual the DNA molecule of claim 1 in an amount effective to induce an immune response against hepatitis C virus, wherein antibodies are produced.

5. A recombinant DNA molecule comprising a nucleotide sequence that encodes a fusion protein, wherein said fusion protein consists of a hepatitis B virus S gene product linked to amino acids 1-70 to 1-154 of the hepatitis C virus core protein.

6. The recombinant nucleic acid molecule of claim 5 wherein the N terminal amino acid of said hepatitis C virus core protein is linked to the C terminal amino acid of said hepatitis B virus S gene product.

7. A method of inducing an immune response against hepatitis C virus in an individual uninfected by hepatitis C virus comprising the step of: administering to said individual the DNA molecule of claim 5 in an amount effective to induce an immune response against hepatitis C virus.

8. A method of treating an individual who is infected with hepatitis C virus comprising the step of: administering to said individual the DNA molecule of claim 5 in an amount effective to induce an immune response against hepatitis C virus, wherein antibodies are produced.

9. A recombinant DNA molecule comprising a nucleotide sequence that encodes a fusion protein, wherein said fusion protein consists of a fragment of the hepatitis B virus pre S2 gene product linked to the hepatitis B virus S gene product linked to amino acids 1-69 of the hepatitis C virus core protein.

10. The recombinant nucleic acid molecule of claim 9 wherein the N terminal amino acid of said hepatitis B virus S gene product is linked to the C terminal amino acid of said hepatitis B virus pre S2 gene product, and the N terminal amino acid of said hepatitis C virus core protein is linked to the C terminal amino acid of said hepatitis B virus S gene product.

11. A method of inducing an immune response against hepatitis C virus in an individual uninfected by hepatitis C virus comprising the step of: administering to said individual the DNA molecule of claim 9 in an amount effective to induce an immune response against hepatitis C virus.

12. A method of treating an individual who is infected with hepatitis C virus comprising the step of: administering to said individual the DNA molecule of claim 9 in an amount effective to induce an immune response against hepatitis C virus, wherein antibodies are produced.

13. A recombinant DNA molecule comprising a nucleotide sequence that encodes a fusion protein, wherein said fusion protein consists of a fragment of the hepatitis B virus pre S2 gene product linked to the hepatitis B virus S gene product linked to amino acids 1-70 to 1-154 of the hepatitis C virus core protein.

14. The recombinant nucleic acid molecule of claim 13 wherein the N terminal amino acid of said hepatitis B virus S gene product is linked to the C terminal amino acid of said hepatitis B virus pre S2 gene product, and the N terminal amino acid of said hepatitis C virus core protein is linked to the C terminal amino acid of said hepatitis B virus S gene product.

15. A method of inducing an immune response against hepatitis C virus in an individual uninfected by hepatitis C virus comprising the step of: administering to said individual the DNA molecule of claim 13 in an amount effective to induce an immune response against hepatitis C virus.

16. A method of treating an individual who is infected with hepatitis C virus comprising the step of: administering to said individual the DNA molecule of claim 13 in an amount effective to induce an immune response against hepatitis C virus, wherein antibodies are produced.

17. A recombinant DNA molecule comprising a nucleotide sequence that

encodes a fusion protein, wherein said fusion protein consists of the hepatitis B virus pre S2 gene product linked to the hepatitis B virus S gene product linked to amino acids 1-69 of the hepatitis C virus core protein.

18. The recombinant nucleic acid molecule of claim 17 wherein the N terminal amino acid of said hepatitis B virus S gene product is linked to the C terminal amino acid of said hepatitis B virus pre S2 gene product, and the N terminal amino acid of said hepatitis C virus core protein is linked to the C terminal amino acid of said hepatitis B virus S gene product.

19. A method of immunizing an individual susceptible to or infected by hepatitis C virus for producing antibodies comprising the step of: administering to said individual the DNA molecule of claim 17 in an amount effective to induce an immune response, wherein antibodies are produced.

20. A method of treating an individual who is infected with hepatitis C virus comprising the step of: administering to said individual the DNA molecule of claim 17 in an amount effective to induce an immune response against hepatitis C virus, wherein antibodies are produced.

21. A recombinant DNA molecule comprising a nucleotide sequence that encodes a fusion protein, wherein said fusion protein consists of the hepatitis B virus pre S2 gene product linked to the hepatitis B virus S gene product linked to amino acids 1-70 to 1-154 of the hepatitis C virus core protein.

22. The recombinant nucleic acid molecule of claim 21 wherein the N terminal amino acid of said hepatitis B virus S gene product is linked to the C terminal amino acid of said hepatitis B virus pre S2 gene product, and the N terminal amino acid of said hepatitis C virus core protein is linked to the C terminal amino acid of said hepatitis B virus S gene product.

23. A method of immunizing an individual susceptible to or infected by hepatitis C virus for producing antibodies comprising the step of: administering to said individual the DNA molecule of claim 21 in an amount effective to induce an immune response, wherein antibodies are produced.

24. A method of treating an individual who is infected with hepatitis C virus comprising the step of: administering to said individual the DNA molecule of claim 21 in an amount effective to induce an immune response against hepatitis C virus, wherein antibodies are produced.

25. The recombinant DNA molecule of any one of claims 1, 5, 9, 13, 17, or 21 comprising a **cytomegalovirus** promoter, a Rous Sarcoma Virus enhancer, and a polyadenylation sequence, wherein the nucleotide coding sequence is operatively linked to said promoter, enhancer, and polyadenylation sequence.

26. The recombinant DNA molecule of claim 25 further comprising the 5' UTR of hepatitis C virus, wherein said nucleotide coding sequence is operatively linked thereto.

27. A recombinant DNA molecule comprising a nucleotide coding sequence that encodes a fusion protein, wherein said fusion protein is selected from the group consisting of: a fusion protein that consists of the hepatitis B virus S gene product linked to a truncated hepatitis C virus core protein, a fusion protein that consists of a fragment of the the hepatitis B virus pre S2 gene product linked to the hepatitis B virus S gene product linked to a truncated hepatitis C virus core protein, and a fusion protein that consists of the hepatitis B virus pre S2 gene product linked to the hepatitis B virus S gene product linked to a

DETD A **DNA vaccine** can be delivered in a non-invasive manner to a variety of susceptible tissue types in order to achieve the desired. . .

DETD Gene gun-based **DNA immunization** achieves direct, intracellular delivery of DNA, elicits higher levels of protective immunity, and requires approximately three orders of magnitude less. . .

DETD The term transfected is used herein to refer to cells which have incorporated the delivered foreign **DNA vaccine**, whichever delivery technique is used.

DETD It is herein disclosed that when inducing cellular, humoral, and protective immune repsonses after **DNA vaccination** the preferred target cells are epidermal cells, rather than cells of deeper skin layers such as the dermis. Epidermal cells are preferred recipients of **DNA vaccines** because they are the most accessible cells of the body and may, therefore, be immunized non-invasively. Secondly, in addition to eliciting a humoral immune response, **DNA immunized** epidermal cells also elicit a cytotoxic immune response that is stronger than that generated in sub-epidermal cells. Delivery to epidermis. . .

DETD . . . TBE. Mice have been used extensively as the laboratory model of choice for assessment of protective immune responses to tick-borne **flaviviruses** (Gajdosova, E. et al.,1981, Acta Virol. 25:10; Heinz, F. X. and C. Kunz, 1982, J. Biol. Stand. 10:25; Holzmann, H.. . .

DETD Generally, the **DNA vaccine** administered may be in an amount of about 1-5 ug of DNA per dose and will depend on the subject. . .

DETD . . . Shope, Yale Arbovirus Research Unit, New Haven, Conn. Cell lines were obtained from the American Type Culture Collection. Central European **encephalitis virus**, strain Hypr, was isolated originally in 1953 from a TBE patient in Czechoslovakia. Russian spring summer **encephalitis virus**, strain Sofjin, was isolated originally in 1937 from a TBE patient from the Far Eastern USSR. Langat virus was isolated. . .

DETD . . . were modified around the translation initiation codon (bold type below) to generate sequences with a favorable context for translation initiation (**Kozak**, M., 1989, J. Cell. Biol. 108:229). The forward and reverse primers for RSSE were: 5'GCAGTAGACAGGATGGGTTGGTTG3' (SEQ ID NO:3) and 5'GCACAGCCAACTTAAGCTCCCACTCC3'. . .

DETD . . . RSSE or CEE prM/E cloned into pWRG7077 (FIG. 1). The two plasmids have the same control elements; i.e., a human **cytomegalovirus** early promoter and intron A, and a bovine growth hormone polyadenylation/transcription termination signal. However, pWRG7077 does not contain the SV40. . .

DETD . . . not as concentrated as the RSSE antigen, in that titers were uniformly lower with sera from both RSSE and CEE **DNA-immunized** mice (FIG. 4).

DETD To determine if the **DNA vaccines** could protect mice from challenge with virulent RSSE and CEE viruses, mice from each of the three experiments described above. . .

DETD TABLE 1

Mortality of mice immunized with RSSE, CEE, or RSSE and CEE naked **DNA vaccines** and challenged with RSSE or CEE viruses

		No. dead/total no.		
Virus(es) used for Vaccine	Challenge Virus	Replicate 1	Replicate 2	Overall
		Vaccinated. . .		

DETD Neutralizing antibodies correlate with protective immunity to tick-borne **flaviviruses**, as demonstrated in mice by passive transfer of neutralizing monoclonal antibodies to M and E (Heinz, F. X. et al.,. . .

DETD We have performed a comprehensive evaluation of **DNA vaccines** for RSSE and CEE viruses in mice (Schmaljohn, C. S. et al, 1997, J. Virol. 71:9563). We demonstrated that high. . . achieve both homologous and heterologous protection of mice from challenge with RSSE and CEE viruses with either of the 2 **DNA vaccines**. Following these successes in mice and prior to human trials, we show that neutralizing antibodies are present following vaccination in. . .

DETD . . . 5 animals were vaccinated with the DNA carrier plasmid (WRG7077) without any gene inserts and served as the negative control. **DNA vaccinated** animals in groups 1, 2, and 5 were immunized with

approximately 10 ug of DNA on days 0, 28, and. . .

DETD After 3 immunizations, sera from monkeys receiving the combination of RSSE and CEE **DNA vaccines** had ELISA titers (on RSSE and CEE antigens) and neutralizing antibody titers (to CEE virus) equivalent to those elicited by. . .

DETD . . . neutralizing antibodies are known to be a correlate of protective immunity, these studies indicate that it is likely that the **DNA vaccine** will protect humans from tick-borne encephalitis caused by RSSE and CEE.

DETD . . . 1992, Virology 187:290). Such subviral particles, consisting of heterodimers of prM and E, are also a by product of normal **flavivirus** morphogenesis; i.e., the so-called "slowly sedimenting hemagglutinins" (SHA) (Heinz, F. and C. Kunz, 1977, Acta Virol. 21:308; Mason, P. W.. . . and P. W. Mason, 1993, supra). So, although passively transferred neutralizing monoclonal antibodies to E can protect animals from subsequent **flavivirus** challenge (Buckley, A. and E. A. Gould, 1985, supra; Gould and Buckley, 1986, supra; Heinz, F. X. et al., 1983,. . .

DETD . . . above, neutralizing antibodies to E are, by themselves, sufficient to protect mice, and presumably humans, from CEE virus. Thus, although **DNA vaccines** delivered to the epidermis by gene gun inoculation efficiently induce both cell-mediated and humoral immune responses (Haynes et al., 1994,. . .

DETD . . . (Holzmann et al., 1997, J. Gen. Virol. 78:31, supra; Holzmann et al., 1992, Vaccine 10:345). Consequently, although either of our **DNA vaccines** by itself may be sufficient for immunity to TBE-causing **flaviviruses**, it may be prudent to include both DNAs in a vaccine developed for humans.

DETD . . . after challenge of some of the mice in our duration of immunity experiments. Additionally, we show that monkeys receiving the **DNA vaccine** had ELISA titers on RSSE and CEE antigens and neutralizing antibody titers to CEE virus equivalent to the commercially available inactivated virus vaccine. Since neutralizing antibodies correlate with protective immunity, the **DNA vaccine** described here is likely to protect humans from tick-borne encephalitis caused by RSSE and CEE.

CLM What is claimed is:

1. A method for inducing a protective immune response to a tick-borne **flavivirus** protein in a mammal, comprising (i) preparing a nucleic acid encoding an antigenic determinant of a tick-borne **flavivirus** prM/E protein operatively linked to a **CMV** promoter operative in cells of a mammal, which nucleic acid is suitable for stably producing the antigenic determinant in a. . . of the mammal in vivo; and (iv) inducing a protective immune response in said mammal upon exposure to a tick-borne **flavivirus**.

3. The method according to claim 1 wherein the tick-borne **flavivirus** prM/E protein is selected from the group consisting of Russian spring summer encephalitis prM/E proteins, and Central European encephalitis prM/E. . .

5. A method for inducing a protective immune response to a tick-borne **flavivirus** protein in a mammal, comprising (i) preparing a nucleic acid encoding an antigenic determinant of a Russian spring summer encephalitis tick-borne **flavivirus** prM/E protein operatively linked to a promoter operative in cells of a mammal, which nucleic acid encodes a protein coding. . . of the mammal in vivo; and (iv) inducing a protective immune response in said mammal upon exposure to a tick-borne **flavivirus**.

7. A kit for inducing a protective immune response to a tick-borne **flavivirus** protein in a mammal, comprising packaged in association: (a) a nucleic acid encoding an antigenic determinant of a tick-borne **flavivirus** prM/E protein operatively linked to a **CMV** promoter operative in cells of a mammal, which nucleic acid is suitable for stably producing the antigenic determinant in a. . .

8. The kit of claim 7, wherein the tick-borne **flavivirus** prM/E protein is selected from the group consisting of Russian spring summer encephalitis prM/E proteins, and Central European encephalitis prM/E. .

.
11. A kit for inducing a protective immune response to a tick-borne **flavivirus** protein in a mammal, comprising packaged in association: (a) a nucleic acid encoding an antigenic determinant of a Russian spring summer encephalitis tick-borne **flavivirus** prM/E protein operatively linked to a promoter operative in cells of a mammal which nucleic acid encodes a protein coding. . .

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=> d l33,cbib,ab,kwic,2-6

L33 ANSWER 2 OF 6 USPATFULL on STN
2000:18426 Chimeric hepatitis B/hepatitis C virus vaccine.
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Wakita, Takaji, Tokyo, Japan
The General Hospital Corporation, Charlestown, MA, United States (U.S. corporation)
US 6025341 20000215
APPLICATION: US 1997-854531 19970512 (8) <--
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Nucleic acid molecule that comprise an incomplete hepatitis C and hepatitis B viral genome including specifically disclosed DNA sequences are disclosed. Pharmaceutical compositions that contain nucleic acid molecules comprising an incomplete hepatitis C and hepatitis B viral genome including a nucleotide sequence encoding a complete hepatitis C core protein and hepatitis B S gene protein operably linked to regulatory elements functional in human cells are disclosed. Methods of immunizing individuals susceptible to or infected by hepatitis B virus and/or hepatitis C virus comprising the step of administering such pharmaceutical compositions are disclosed.

AI US 1997-854531 19970512 (8) <--

SUMM . . . relates to recombinant chimeric gene constructs which are useful as anti-hepatitis B virus and/or anti-hepatitis C virus vaccine components in **genetic immunization** protocols, to methods of protecting individuals against hepatitis B virus and/or hepatitis C virus infection and to methods of treating. . .

SUMM . . . positive stranded RNA virus, approximately 9,500 nucleotides in length, which has recently been classified as a separate genus within the **Flavivirus** family (Heinz, F. X., Arch. Virol. (Suppl.), 1992, 4, 163-171). Different isolates show considerable nucleotide sequence diversity leading to the. . .

SUMM . . . Ser. No. PCT/US94/00899 filed Jan. 26, 1994, and U.S. Ser. No. 08/221,579 filed Apr. 1, 1994 each contains descriptions of **genetic immunization** protocols. Vaccines against HCV are disclosed in each.

DETD It has been shown that many proteins previously known to induce a humoral and cellular immune responses following **DNA immunization** have either been native cell surface proteins or secreted proteins, such as, for example, influenza NP, HBsAg, and rabies virus. . .

DETD . . . of directing expression in the cells of the vaccinated individual. In some embodiments, the gene construct further comprises an enhancer, **Kozak** sequence (GCCGCCATG SEQ ID NO:13), and at least a fragment of the HCV 5' UTR.

DETD . . . The regulatory elements include a promoter and a polyadenylation signal. In addition, other elements, such as an enhancer and a **Kozak** sequence, may also be included in the gene construct.

DETD . . . Tumor Virus (MMTV) promoter, Human Immunodeficiency Virus (HIV) such as the HIV Long Terminal Repeat (LTR) promoter, Moloney virus, ALV, **Cytomegalovirus** (**CMV**) such as the **CMV immediate early promoter**, Epstein Barr Virus (EBV), Rous Sarcoma Virus (RSV) as well as promoters from human genes such as human Actin, human. . .

DETD . . . but not limited to: human Actin, human Myosin, human Hemoglobin, human muscle creatine and viral enhancers such as those from **CMV**, RSV and EBV.

DETD In expression vectors of the invention, nucleotide coding sequence encoding the fusion protein is under the regulatory control of the **CMV immediate early promoter** and the SV40 minor polyadenylation signal. Constructs may optionally contain the SV40 origin of replication.

DETD . . . (pre S2-S) PCR product by Xho-I followed by Klenow treatment. In the upstream sequence of the pre-S2-S-HCV fusion constructs, a **Kozak** sequence (GCCGCCATG SEQ ID NO:13) was included in the Kz Hind pS2 primer and this was added to the preS2-S-HCV. . .

DETD . . . proteins described above each contain the nucleotide coding region for the fusion protein placed under the transcriptional control of the **CMV** promoter and the RSV enhancer element.

DETD . . . and operably linked to the promoter and polyadenylation signal. Transcription of the cloned inserts is under the control of the **CMV** promoter and the RSV enhancer elements. A polyadenylation signal is provided by the presence of an SV40 poly A signal. . .

CLM What is claimed is:

25. The recombinant DNA molecule of any one of claims 1, 5, 9, 13, 17, or 21 comprising a **cytomegalovirus** promoter, a Rous Sarcoma Virus enhancer, and a polyadenylation sequence, wherein the nucleotide coding sequence is operatively linked to said. . .

32. The pharmaceutical composition of claim 31 wherein said regulatory elements functional in human cells comprise a **cytomegalovirus** promoter, a Rous Sarcoma Virus enhancer, a polyadenylation sequence.

37. The pharmaceutical composition of claim 36 wherein said regulatory elements functional in human cells comprise a **cytomegalovirus** promoter, a Rous Sarcoma Virus enhancer, a polyadenylation sequence.

42. The pharmaceutical composition of claim 41 wherein said regulatory elements functional in human cells comprise a **cytomegalovirus** promoter, a Rous Sarcoma Virus enhancer, a polyadenylation sequence.

47. The pharmaceutical composition of claim 46 wherein said regulatory elements functional in human cells comprise a **cytomegalovirus** promoter, a Rous Sarcoma Virus enhancer, a polyadenylation sequence.

52. The pharmaceutical composition of claim 51 wherein said regulatory elements functional in human cells comprise a **cytomegalovirus** promoter, a Rous Sarcoma Virus enhancer, a polyadenylation sequence.

57. The pharmaceutical composition of claim 56 wherein said regulatory elements functional in human cells comprise a **cytomegalovirus** promoter, a Rous Sarcoma Virus enhancer, a polyadenylation sequence.

L33 ANSWER 3 OF 6 USPATFULL on STN

1999:141912 Compositions and methods for delivery of genetic material.

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US 5981505 19991109
WO 9416737 19940804
APPLICATION: US 1997-979385 19971126 (8) <--
WO 1994-US899 19940126 19950828 PCT 371 date 19950828 PCT 102(e) date<--
DOCUMENT TYPE: Utility; Granted.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Methods of inducing genetic material into cells of an individual and compositions and kits for practicing the same are disclosed. The methods comprise the steps of contacting cells of an individual with a polynucleotide function enhancer and administering to the cells, a nucleic acid molecule that is free of retroviral particles. The nucleic acid molecule comprises a nucleotide sequence that encodes a protein

that comprises at least one epitope that is identical or substantially similar to an epitope of a pathogen antigen or an antigen associated with a hyperproliferative or autoimmune disease, a protein otherwise missing from the individual due to a missing, non-functional or partially functioning gene, or a protein that produces a therapeutic effect on an individual. Methods of prophylactically and therapeutically immunizing an individual against HIV are disclosed. Pharmaceutical compositions and kits for practicing methods of the present invention are disclosed.

AI US 1997-979385 19971126 (8) <--
WO 1994-US899 19940126 <--
19950828 PCT 371 date
19950828 PCT 102(e) date

DETD . . . individual. Regulatory elements for DNA expression include a promoter and a polyadenylation signal. In addition, other elements, such as a **Kozak** region, may also be included in the genetic construct.

DETD . . . Tumor Virus (MMTV) promoter, Human Immunodeficiency Virus (HIV) such as the HIV Long Terminal Repeat (LTR) promoter, Moloney virus, ALV, **Cytomegalovirus** (**CMV**) such as the **CMV immediate early promoter**, Epstein Barr Virus (EBV), Rous Sarcoma Virus (RSV) as well as promoters from human genes such as human Actin, human. . .

DETD . . . but not limited to: human Actin, human Myosin, human Hemoglobin, human muscle creatine and viral enhancers such as those from **CMV**, RSV and EBV.

DETD . . . the present invention are useful in the fields of both human and veterinary medicine. Accordingly, the present invention relates to **genetic immunization** of mammals, birds and fish. The methods of the present invention can be particularly useful for mammalian species including human,. . .

DETD The present invention provides an HIV vaccine using direct **genetic immunization**. Genetic constructs are provided which, when delivered into the cells of an individual, are expressed to produce HIV proteins. According. . .

DETD . . . and a polyadenylation signal. The promoter may be selected from the group consisting of: HIV LTR, human Actin, human Myosin, **CMV**, RSV, Moloney, MMTV, human Hemoglobin, human muscle creatine and EBV. The enhancer may be selected from the group consisting of: human Actin, human Myosin, **CMV**, RSV, human Hemoglobin, human muscle creatine and EBV. The polyadenylation signal may be selected from the group consisting of: LTR. . .

DETD . . . HXB2 was amplified via PCR and cloned into the expression vector pCNDA/neo (Invitrogen). This plasmid drives envelope production through the **CMV** promoter.

DETD In the **genetic immunization** procedure described herein, the quadriceps muscles of BALB/c mice were injected with 100 μl of 0.5% bupivacaine-HCl and 0.1% methylparaben. . .

DETD To determine whether the antisera generated by **DNA immunization** possessed antiviral activity, the ability of the antisera to neutralize HIV-1 infection was examined. Cell-free HIV-1/III_B virus at 100 $TCID_{50}$. . .

DETD . . . isotypes indicates that a secondary immune response has taken place, and further suggests that helper T-cells can be elicited by **genetic immunization**.

DETD In the **genetic immunization** procedure described herein, the quadriceps muscles of BALB/c mice were injected with 100 μl of 0.5% bupivacaine-HCl and 0.1% methylparaben. . .

DETD . . . expression is MMTV LTR. The promoter may be deleted and replaced with Actin promoter, myosin promoter, HIV LTR promoter and **CMV** promoter.

DETD . . . Actin no no yes
RA-4 Actin CME yes yes
RA-5 Actin CME yes no
RA-6 Actin CME no yes
RA-7 **CMV** no yes yes
RA-8 **CMV** no yes no
RA-9 **CMV** no no yes

RA-10 **CMV** CME yes yes
RA-11 **CMV** CME yes no
RA-12 **CMV** CME no yes
RA-13 MMTV no yes yes
RA-14 MMTV no yes no
RA-15 MMTV no no yes
RA-16 MMTV. . .

DETD . . . The HIV 5'LTR promoter can be deleted and replaced with Moloney virus promoter, MMTV LTR, Actin promoter, myosin promoter and **CMV** promoter.

DETD . . . Moloney HIV 3' LTR yes
LA-2 Moloney SV40 yes
LA-3 Moloney HIV 3' LTR no
LA-4 Moloney SV40 no
LA-5 **CMV** HIV 3' LTR yes
LA-6 CNV SV40 yes
LA-7 **CMV** HIV 3' LTR no
LA-8 **CMV** SV40 no
LA-9 MMTV HIV 3' LTR yes
LA-10 MMTV SV40 yes
LA-11 MMTV HIV 3' LTR no
LA-12 MMTV. . .

DETD . . . the thymidine kinase promoter and polyadenylation site. The HIV env coding region is placed under the regulatory control of the **CMV** promoter and SV40 polyadenylation site. The HIV env coding region was obtained as a 2.3 kb PCR fragment form HIV/3B,. . .

DETD . . . the thymidine kinase promoter and polyadenylation site. The HIV gag/pol coding region is placed under the regulatory control of the **CMV** promoter and SV40 polyadenylation site. The HIV gag/pol coding region was obtained from HIV MN, Genebank sequence MI7449, and includes. . .

DETD . . . the thymidine kinase promoter and polyadenylation site. The HIV gag/pol coding region is placed under the regulatory control of the **CMV** promoter and SV40 polyadenylation site. The HIV gag/pol coding region was obtained from HIV MN, Genebank sequence MI7449, and includes. . .

DETD . . . includes a Kanamycin resistance gene and a pBR322 origin of DNA replication. The sequences provided for transcription regulation include: a **cytomegalovirus** promoter; a Rous sarcoma virus enhancer; and an SV40 polyadenylation signal. The HIV-1 sequences included in pGAGPOL.rev include a sequence. . .

DETD Several safety features are included in pGAGPOL.rev. These include use of the **CMV** promoter and a non-retroviral poly(A) site. Furthermore, deletion of the ψ sequence limits the ability to package viral RNA. In. . .

DETD . . . includes a Kanamycin resistance gene and a pBR322 origin of DNA replication. The sequences provided for transcription regulation include: a **cytomegalovirus** promoter; a Rous sarcoma virus enhancer; and an SV40 polyadenylation signal. The HIV-1 sequences included in pENV include a sequence. . .

DETD Several safety features are included in PGAGPOL.rev. These include use of the **CMV** promoter and a non-retroviral poly(A) site. Furthermore, tat has been deleted and a 50% deletion of nef yields an "inactive". .

DETD Step 4a. Digest with BamHI and ligate with the **CMV** promoter obtained by PCR of pCEP4 (Invitrogen, San Diego Calif.) with primers SEQ ID NO:25 and SEQ ID NO:26.

DETD . . . be any promoter functional in a human cell. It is preferred that the promoter is an SV40 promoter or a **CMV** promoter, preferably a **CMV immediate early promoter**. The polyadenylation signal may be any polyadenylation signal functional in a human cell. It is preferred that the polyadenylation signal. . .

DETD . . . be any promoter functional in a human cell. It is preferred that the promoter is an SV40 promoter or a **CMV** promoter, preferably a immediate early **CMV** promoter. The polyadenylation signal may be any polyadenylation signal functional in a human cell. It is preferred that

the polyadenylation. . .

DETD . . . identical to or substantially similar to epitopes of HIV proteins. The coding sequence is under the regulatory control of the **CMV immediate early promoter** and the SV40 minor polyadenylation signal.

DETD . . . encodes at least one HIV protein or a fragment thereof. The coding sequence is under the regulatory control of the **CMV immediate early promoter** and the SV40 minor polyadenylation signal. The HIV protein is selected from the group consisting of gag, pol, env and. . .

DETD . . . identical to or substantially similar to epitopes of HIV proteins. The coding sequence is under the regulatory control of the **CMV immediate early promoter** and the SV40 minor polyadenylation signal. The two expression units are encoded in opposite directions of each other.

DETD . . . protein or a fragment thereof. Each expression unit comprises a coding sequence that is under the regulatory control of the **CMV immediate early promoter** and the SV40 minor polyadenylation signal. The HIV protein is selected from the group consisting of gag, pol, env and. . .

DETD Step 9. Cut pCEP4 (Invitrogen) with SalI to release a DNA fragment containing the **CMV** promoter, polylinker, and SV40 poly A site. Purify this fragment and blunt-off with the Klenow fragment of DNA Polymerase I.

DETD . . . fragment obtained in step 9. Isolate plasmid containing the bacterial origin of replication, the Kan^R gene, the RSV enhancer, the **CMV** promoter, polylinker, and the SV40 poly A site.

DETD **Genetic Immunization** with Genes for Regulatory Proteins

DETD . . . regulatory and/or enzymatic proteins, rather than the entire complement of HIV genes. Accordingly, a focused immunization strategy may desirably involve **genetic immunization** using coding sequences for one or more regulatory, non-structural HIV proteins, including tat, rev, vpr, nef, vpu or vif. only. . .

DETD In some embodiments of **genetic immunization** against HIV using regulatory genes, the one or more of tat, rev, nef, vif and vpu genes are inserted into. . .

DETD . . . in HIV-infected individuals. Because of such potentially detrimental effects in both HIV-infected and -noninfected individuals, preferred tat constructs employed for **genetic immunization** are modified to express only non-functional Tat. Mutations capable of inactivating Tat or Rev can in addition act as transdominant. . .

DETD . . . may be responsible for the muscle wasting frequently observed in AIDS patients. Because of the potentially detrimental activity of Vpr, **genetic immunization** should preferably be carried out with a modified vpr construct which will express a non-functional Vpr protein.

DETD . . . much like Tat and also exhibits vpr-like activity) and Rex (which acts much like Rev) is cleared in many individuals. **Genetic immunization** with regulatory genes is considered relevant not only for HIV, but also for viruses such as HBV (X gene product). . .

DETD . . . AvaII and which contains a kanamycin resistance gene and a pBR322 origin of replication. In addition, this plasmid contains a **cytomegalovirus** promoter, a Rous sarcoma virus enhancer, the rev coding region and a SV40 polyadenylation signal. The rev sequence present in. . .

DETD **Genetic Immunization** with Enzymatic Genes

DETD **Genetic immunization** with genes encoding proteins with enzymatic functions, such as the HIV pol gene can also be an important antiviral strategy. . . is non-pathogenic and non-infectious. Similarly, the enzymatic genes of other viruses, such as the HBV polymerase, are attractive targets for **genetic immunization**. See, e.g., Radziwill et al., Mutational Analysis of the Hepatitis B Virus P Gene Product: Domain Structure and RNase H. . .

DETD . . . into a plasmid containing a kanamycin resistance gene and a pBR322 origin of replication. In addition, this plasmid contains a **cytomegalovirus** promoter, a Rous sarcoma virus enhancer, and a SV40 polyadenylation signal. The translation initiation codons for surface antigen and the. . .

DETD . . . into a vector containing a kanamycin resistance gene and a
pBR322 origin of replication. In addition, this plasmid contains a
cytomegalovirus promoter, a Rous sarcoma virus enhancer, and a SV40
polyadenylation signal. The 5' PCR primer for this amplification
contains a. . . After ligation of this PCR product into a plasmid
containing the kanamycin resistance gene, a pBR322 origin of
replication, a **cytomegalovirus** promoter, a Rous sarcoma virus
enhancer, and a SV40 polyadenylation signal, the translation initiation
codons for the Hepatitis B surface. . .

DETD . . . include Senilis viruses,
RossRiver virus and Eastern & Western
Equine encephalitis.
Reovirus: (Medical) Rubella virus.
Flariviridue Family
Examples include: (Medical) **dengue**,
yellow fever, Japanese encephalitis, St.
Louis encephalitis and tick borne
encephalitis viruses.
Hepatitis C Virus: (Medical) these viruses are not placed in
a family yet but are believed to be either a togavirus or a
flavivirus. Most similarity is with togavirus family.
Coronavirus Family:
(Medical and Veterinary)
Infectious bronchitis virus (poultry)
Porcine transmissible gastroenteric virus
(pig)
Porcine. . . Herpesvirus Family
Sub-Family: alphaherpesviridue
Genera: Simplexvirus (Medical)
HSVI, HSVII
Varicellovirus: (Medical - Veterinary)
pseudorabies - varicella zoster
Sub-Family
betaherpesviridue
Genera: **Cytomegalovirus** (Medical)
HCMV
Muromega lovirus
Sub-Family: Gammaherpesviridue
Genera: Lymphocryptovirus (Medical)
EBV - (Burkitts lympho)
Rhadinovirus
Poxvirus Family
Sub-Family: Chordopoxviridue (Medical -. . .

CLM What is claimed is:

. . . hepatitis B virus; hepatitis C virus; human papilloma virus, HPV;
Herpes simplex 1 virus, HSV1; Herpes simplex 2 virus, HSV2;
Cytomegalovirus, **CMV**; Epstein-Barr virus, EBR; rhinovirus; and,
coronavirus.

. . . hepatitis B virus; hepatitis C virus; human papilloma virus, HPV;
Herpes simplex 1 virus, HSV1; Herpes simplex 2 virus, HSV2;
Cytomegalovirus, **CMV**; Epstein-Barr virus, EBR; rhinovirus; and,
coronavirus.

. . . hepatitis B virus; hepatitis C virus; human papilloma virus, HPV;
Herpes simplex 1 virus, HSV1; Herpes simplex 2 virus, HSV2;
Cytomegalovirus, **CMV**; Epstein-Barr virus, EBR; rhinovirus; and,
coronavirus.

L33 ANSWER 4 OF 6 USPATFULL on STN

1999:121330 Compositions and methods for delivery of genetic material.

Carrano, Richard A., Paoli, PA, United States
Wang, Bin, Haidian, China
Weiner, David B., Merion, PA, United States
Apollon, Inc., Malvern, PA, United States (U.S. corporation)The Trustees Of

The University of Pennsylvania, Philadelphia, PA, United States (U.S. corporation)
US 5962428 19991005
WO 9526718 19951012
APPLICATION: US 1996-704701 19960916 (8) <--
WO 1995-US4071 19950330 19960916 PCT 371 date 19960916 PCT 102(e) date<--
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Methods of introducing genetic material into cells of an individual and compositions and kits for practicing the same are disclosed. The methods comprise the steps of contacting cells of an individual with a genetic vaccine facilitator and administering to the cells a nucleic acid molecule that is free of retroviral particles. The nucleic acid molecule comprises a nucleotide sequence that encodes a protein that comprises at least one epitope that is identical or substantially similar to an epitope of a pathogen antigen or an antigen associated with a hyperproliferative or autoimmune disease, a protein otherwise missing from the individual due to a missing, non-functional or partially functioning gene, or a protein that produces a therapeutic effect on an individual. Methods of prophylactically and therapeutically immunizing an individual against HIV are disclosed. Pharmaceutical compositions and kits for practicing methods of the present invention are disclosed.
AI US 1996-704701 19960916 (8) <--
WO 1995-US4071 19950330 <--
19960916 PCT 371 date
19960916 PCT 102(e) date
DETD . . . individual. Regulatory elements for DNA expression include a promoter and a polyadenylation signal. In addition, other elements, such as a **Kozak** region, may also be included in the genetic construct.
DETD . . . Tumor Virus (MMTV) promoter, Human Immunodeficiency Virus (HIV) such as the HIV Long Terminal Repeat (LTR) promoter, Moloney virus, ALV, **Cytomegalovirus** (**CMV**) such as the **CMV immediate early promoter**, Epstein Barr Virus (EBV), Rous Sarcoma Virus (RSV) as well as promoters from human genes such as human Actin, human. . .
DETD . . . but not limited to: human Actin, human Myosin, human Hemoglobin, human muscle creatine and viral enhancers such as those from **CMV**, RSV and EBV.
DETD . . . the present invention are useful in the fields of both human and veterinary medicine. Accordingly, the present invention relates to **genetic immunization** of mammals, birds and fish. The methods of the present invention can be particularly useful for mammalian species including human,. . .
DETD The present invention provides an HIV vaccine using direct **genetic immunization**. Genetic constructs are provided which, when delivered into the cells of an individual, are expressed to produce HIV proteins. According. . .
DETD . . . and a polyadenylation signal. The promoter may be selected from the group consisting of: HIV LTR, human Actin, human Myosin, **CMV**, RSV, Moloney, MMTV, human Hemoglobin, human muscle creatine and EBV. The enhancer may be selected from the group consisting of: human Actin, human Myosin, **CMV**, RSV, human Hemoglobin, human muscle creatine and EBV. The polyadenylation signal may be selected from the group consisting of: LTR. . .
DETD . . . HXB2 was amplified via PCR and cloned into the expression vector pCNDA/neo (Invitrogen). This plasmid drives envelope production through the **CMV** promoter.
DETD . . . expression is MMTV LTR. The promoter may be deleted and replaced with Actin promoter, myosin promoter, HIV LTR promoter and **CMV** promoter.
DETD . . . Actin no yes no
RA-3 Actin no no yes
RA-4 Actin CME yes yes
RA-5 Actin CME yes no
RA-6 Actin CME no yes
RA-7 **CMV** no yes yes
RA-8 **CMV** no yes no

RA-9	CMV	no	no	yes
RA-10	**CMV**	CME	yes	yes
RA-11	**CMV**	CME	yes	no
RA-12	**CMV**	CME	no	yes
RA-13	MMTV	no	yes	yes
RA-14	MMTV	no	yes	no
RA-15	MMTV	no	no	yes
RA-16	MMTV	CME	yes	yes
RA-17	MMTV. . .			

DETD . . . The HIV 5'LTR promoter can be deleted and replaced with Moloney virus promoter, MMTV LTR, Actin promoter, myosin promoter and **CMV** promoter.

DETD

Construct	Promoter	poly(A)	Amp^r
LA-1	Moloney	HIV 3'LTR	yes
LA-2	Moloney	SV40	yes
LA-3	Moloney	HIV 3'LTR	no
LA-4	Moloney	SV40	no
LA-5	**CMV**	HIV 3'LTR	yes
LA-6	**CMV**	SV40	yes
LA-7	**CMV**	HIV 3'LTR	no
LA-8	**CMV**	SV40	no
LA-9	MMTV	HIV 3'LTR	yes
LA-10	MMTV	SV40	yes
LA-11	MMTV	HIV 3'LTR	no
LA-12	MMTV	SV40	no
LA-13	HIV 5' LTR	HIV. . .	

DETD . . . the thymidine kinase promoter and polyadenylation site. The HIV env coding region is placed under the regulatory control of the **CMV** promoter and SV40 polyadenylation site. The HIV env coding region was obtained as a 2.3 kb PCR fragment form HIV/3B,. . .

DETD . . . the thymidine kinase promoter and polyadenylation site. The HIV gag/pol coding region is placed under the regulatory control of the **CMV** promoter and SV40 polyadenylation site. The HIV gag/pol coding region was obtained from HIV MN, Genebank sequence MI7449, and includes. . .

DETD . . . the thymidine kinase promoter and polyadenylation site. The HIV gag/pol coding region is placed under the regulatory control of the **CMV** promoter and SV40 polyadenylation site. The HIV gag/pol coding region was obtained from HIV MN, Genebank sequence MI7449, and includes. . .

DETD . . . includes a Kanamycin resistance gene and a pBR322 origin of DNA replication. The sequences provided for transcription regulation include: a **cytomegalovirus** promoter; a Rous sarcoma virus enhancer; and an SV40 polyadenylation signal. The HIV-1 sequences included in pGAGPOL.rev include a sequence. . .

DETD Several safety features are included in pGAGPOL.rev. These include use of the **CMV** promoter and a non-retroviral poly(A) site. Furthermore, deletion of the ψ sequence limits the ability to package viral RNA. In. . .

DETD . . . includes a Kanamycin resistance gene and a pBR322 origin of DNA replication. The sequences provided for transcription regulation include: a **cytomegalovirus** promoter; a Rous sarcoma virus enhancer; and an SV40 polyadenylation signal. The HIV-1 sequences included in pENV include a sequence. . .

DETD Several safety features are included in pGAGPOL.rev. These include use of the **CMV** promoter and a non-retroviral poly(A) site. Furthermore, tat has been deleted and a 50% deletion of nef yields an "inactive". . .

DETD Step 4a. Digest with BamHI and ligate with the **CMV** promoter obtained by PCR of pCEP4 (Invitrogen, San Diego Calif.) with primers SEQ ID NO:21 and SEQ ID NQ:22.

DETD . . . be any promoter functional in a human cell. It is preferred that the promoter is an SV40 promoter or a **CMV** promoter, preferably a **CMV immediate early promoter**. The polyadenylation signal may be

any polyadenylation signal functional in a human cell. It is preferred that the polyadenylation signal. . .

DETD . . . be any promoter functional in a human cell. It is preferred that the promoter is an SV40 promoter or a **CMV** promoter, preferably a immediate early **CMV** promoter. The polyadenylation signal may be any polyadenylation signal functional in a human cell. It is preferred that the polyadenylation. . .

DETD . . . identical to or substantially similar to epitopes of HIV proteins. The coding sequence is under the regulatory control of the **CMV immediate early promoter** and the SV40 minor polyadenylation signal.

DETD . . . encodes at least one HIV protein or a fragment thereof. The coding sequence is under the regulatory control of the **CMV immediate early promoter** and the SV40 minor polyadenylation signal. The HIV protein is selected from the group consisting of gag, pol, env and. . .

DETD . . . identical to or substantially similar to epitopes of HIV proteins. The coding sequence is under the regulatory control of the **CMV immediate early promoter** and the SV40 minor polyadenylation signal. The two expression units are encoded in opposite directions of each other.

DETD . . . protein or a fragment thereof. Each expression unit comprises a coding sequence that is under the regulatory control of the **CMV immediate early promoter** and the SV40 minor polyadenylation signal. The HIV protein is selected from the group consisting of gag, pol, env and. . .

DETD Step 9. Cut pCEP4 (Invitrogen) with SalI to release a DNA fragment containing the **CMV** promoter, polylinker, and SV40 poly A site. Purify this fragment and blunt-off with the Klenow fragment of DNA Polymerase I.

DETD . . . fragment obtained in step 9. Isolate plasmid containing the bacterial origin of replication, the Kan^R gene, the RSV enhancer, the **CMV** promoter, polylinker, and the SV40 poly A site.

DETD **Genetic Immunization** with Genes for Regulatory Proteins

DETD . . . regulatory and/or enzymatic proteins, rather than the entire complement of HIV genes. Accordingly, a focused immunization strategy may desirably involve **genetic immunization** using coding sequences for one or more regulatory, non-structural HIV proteins, including tat, rev, vpr, nef, vpu or vif. Only. . .

DETD In some embodiments of **genetic immunization** against HIV using regulatory genes, the one or more of tat, rev, nef, vif and vpu genes are inserted into. . .

DETD . . . in HIV-infected individuals. Because of such potentially detrimental effects in both HIV-infected and -noninfected individuals, preferred tat constructs employed for **genetic immunization** are modified to express only non-functional Tat. Mutations capable of inactivating Tat or Rev can in addition act as transdominant. . .

DETD . . . may be responsible for the muscle wasting frequently observed in AIDS patients. Because of the potentially detrimental activity of Vpr, **genetic immunization** should preferably be carried out with a modified vpr construct which will express a non-functional vpr protein.

DETD . . . much like Tat and also exhibits vpr-like activity) and Rex (which acts much like Rev) is cleared in many individuals. **Genetic immunization** with regulatory genes is considered relevant not only for HIV, but also for viruses such as HBV (X gene product). . .

DETD . . . AvaII and which contains a kanamycin resistance gene and a pBR322 origin of replication. In addition, this plasmid contains a **cytomegalovirus** promoter, a Rous sarcoma virus enhancer, the rev coding region and a SV40 polyadenylation signal. The rev sequence present in. . .

DETD **Genetic Immunization** with Enzymatic Genes

DETD **Genetic immunization** with genes encoding proteins with enzymatic functions, such as the HIV pol gene can also be an important antiviral strategy. . . is non-pathogenic and non-infectious. Similarly, the enzymatic genes of other viruses, such as the HBV polymerase, are attractive targets for **genetic immunization**. See, e.g., Radziwill et al., Mutational Analysis of the Hepatitis B Virus P Gene Product: Domain

Structure and RNase H. . .

DETD . . . into a plasmid containing a kanamycin resistance gene and a pBR322 origin of replication. In addition, this plasmid contains a **cytomegalovirus** promoter, a Rous sarcoma virus enhancer, and a SV40 polyadenylation signal. The translation initiation codons for surface antigen and the. . .

DETD . . . into a vector containing a kanamycin resistance gene and a pBR322 origin of replication. In addition, this plasmid contains a **cytomegalovirus** promoter, a Rous sarcoma virus enhancer, and a SV40 polyadenylation signal. The 5' PCR primer for this amplification contains a. . . After ligation of this PCR product into a plasmid containing the kanamycin resistance gene, a pBR322 origin of replication, a **cytomegalovirus** promoter, a Rous sarcoma virus enhancer, and a SV40 polyadenylation signal, the translation initiation codons for the Hepatitis B surface. . .

DETD . . . examples include Senilis viruses,
RossRiver virus and Eastern & Western
Equine encephaiitis.
Reovirus: (Medical) Rubeila virus.

Flariviridue Family
Examples include: (Medical) **dengue**, yellow fever, Japanese encephalitis, St. Louis encephalitis and tick borne **encephalitis viruses**.

Hepatitis C Virus: (Medical) these viruses are not placed in a family yet but are believed to be either a togavirus or a **flavivirus**. Most similarity is with togavirus family.

Coronavirus Family:
(Medical and Veterinary)
Infectious bronchitis virus (poultry)
Porcine transmissible gastroenteric virus (pig)
Porcine. . . enteritis

Parvovirus Family (Veterinary)
Feline parvovirus: causes feline enteritis
Feline panleucopeniavirus
Canine parvovirus
Porcine parvovirus

Herpesvirus Family
Sub-Family:
alphaherpesviridue
Genera: Simplexvirus (Medical)
HSVI, HSVII
Varicellovirus: (Medical-Veterinary)
pseudorabies-varicella zoster

Sub-Family-betaherpesviridue
Genera: **Cytomegalovirus** (Medical)
HCMV
Muromegalovirus

Sub-Family:
Gammaherpesviridue
Genera: Lymphocryptovirus (Medical)
EBV-(Burkitts lympho)
Rhadinovirus

Poxvirus Family
Sub-Family:
Chordopoxviridue (Medical-Veterinary)
Genera: Variola (Smallpox)
Vaccinia (Cowpox)
Parapoxivirus-Veterinary
Auipoxvirus-Veterinary
Capripoxvirus
Leporipoxvirus

. . .

CLM What is claimed is:

. . . virus, HBV; hepatitis C virus, HCV; human papilloma virus, HPV;

Herpes simplex 1 virus, HSV1; Herpes simplex 2 virus, HSV2; **Cytomegalovirus, CMV**; Epstein-Barr virus, EBV; rhinovirus; and, coronavirus.

L33 ANSWER 5 OF 6 USPATFULL on STN
1998:122388 **Genetic immunization**.
Weiner, David B., Merion, PA, United States
Williams, William V., Havertown, PA, United States
Wang, Bin, Havertown, PA, United States
The Trustees of the University of Pennsylvania, Philadelphia, PA, United States (U.S. corporation)The Wistar Institute, Philadelphia, PA, United States (U.S. corporation)
US 5817637 19981006
APPLICATION: US 1997-783818 19970113 (8) <--
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Methods of prophylactic and therapeutic immunization of an individual against pathogen infection, diseases associated with hyperproliferative cells and autoimmune diseases are disclosed. The methods comprise the steps of administering to cells of an individual, a nucleic acid molecule that comprises a nucleotide sequence that encodes a protein which comprises at least one epitope that is identical or substantially similar to an epitope of a pathogen antigen, a hyperproliferative cell associated protein or a protein associated with autoimmune disease respectively. In each case, nucleotide sequence is operably linked to regulatory sequences to enable expression in the cells. The nucleic acid molecule is free of viral particles and capable of being expressed in said cells. The cells may be contacted cells with a cell stimulating agent. Methods of prophylactically and therapeutically immunizing an individual against HIV are disclosed. Pharmaceutical compositions and kits for practicing methods of the present invention are disclosed.
TI **Genetic immunization**
AI US 1997-783818 19970113 (8) <--
DETD . . . cell surface. The Class I MHC-target antigen complexes are capable of stimulating CD8+ T-cells, which are phenotypically the killer/suppressor cells. **Genetic immunization** according to the present invention is thus capable of eliciting cytotoxic T-cell (CTL) responses (killer cell responses). It has been observed that **genetic immunization** according to the present invention is more likely to elicit CTL responses than other methods of immunization.
DETD . . . elimination of deleterious cell types which, during their production of proteins, display antigens bound by Class I MHC molecules. Therefore, **genetic immunization** according to the present invention is more likely to result in anti-pathogen protection and therapy than standard immunization using killed,. . .
DETD **Genetic immunization** according to the present invention elicits an effective immune response without the use of infective agents or infective vectors. Vaccination. . .
DETD . . . the individual. Regulatory elements for DNA include a promoter and a polyadenylation signal. In addition, other elements, such as a **Kozak** region, may also be included in the genetic construct.
DETD . . . Tumor Virus (MMTV) promoter, Human Immunodeficiency Virus (HIV) such as the HIV Long Terminal Repeat (LTR) promoter, Moloney virus, ALV, **Cytomegalovirus** (**CMV**) such as the **CMV immediate early promoter**, Epstein Barr Virus (EBV), Rous Sarcoma Virus (RSV) as well as promoters from human genes such as human Actin, human. . .
DETD . . . but not limited to: human Actin, human Myosin, human Hemoglobin, human muscle creatine and viral enhancers such as those from **CMV**, RSV and EBV.
DETD . . . non-human as well as human individuals against pathogens and protein specific disorders and diseases. Accordingly, the present invention relates to **genetic immunization** of mammals, birds and fish. The methods of the present invention can be particularly useful for mammalian species including human,. . .
DETD . . . individual. The administration protocols and genecic constructs

useful in gene therapy applications are the same as those described above for **genetic immunization** except the genetic constructs include nucleotide sequences that encode proteins whose presence in the individual will eliminate a deficiency in. . .

DETD The present invention provides an HIV vaccine using direct **genetic immunization**. Genetic constructs are provided which, when delivered into the cells of an individual, are expressed to produce HIV proteins. According. . .

DETD . . . and a polyadenylation signal. The promoter may be selected from the group consisting of: HIV LTR, human Actin, human Myosin, **CMV**, RSV, Moloney, MMTV, human Hemoglobin, human muscle creatine and EBV. The enhancer may be selected from the group consisting of: human Actin, human Myosin, **CMV**, RSV, human Hemoglobin, human muscle creatine and EBV. The polyadenylation signal may be selected from the group consisting of: LTR. . .

DETD . . . HXB2 was amplified via PCR and cloned into the expression vector pCNDA/neo (Invitrogen). This plasmid drives envelope production through the **CMV** promoter.

DETD The following is a description of the use of **genetic immunization** for elicitation of an anti-human immunodeficiency virus type 1 (HIV-1) immune response in mice by administering a DNA construct that. . .

DETD In the **genetic immunization** procedure described herein, the quadriceps muscles of BALB/c mice were injected with 100 µl of 0.5% bupivacaine-HCl and 0.1% methylparaben. . .

DETD To determine whether the antisera generated by **DNA immunization** possessed antiviral activity, the ability of the antisera to neutralize HIV-1 infection was examined. Cell-free HIV-1/III_B virus at 100 $TCID_{50}$. . .

DETD . . . Berman P. W., et al., (1990) Nature 345:622-625. The reasons for the more effective generation of anti-viral activities by the **genetic immunization** than by recombinant protein immunization are not clear. However, the differences in the generated immune responses may be due to. . .

DETD . . . isotypes indicates that a secondary immune response has taken place, and further suggests that helper T-cells can be elicited by **genetic immunization**.

DETD In the **genetic immunization** procedure described herein, the quadriceps muscles of BALB/c mice were injected with 100 µl of 0.5% bupivacaine-HCl and 0.1% methylparaben. . .

DETD . . . expression is MMTV LTR. The promoter may be deleted and replaced with Actin promoter, myosin promoter, HIV LTR promoter and **CMV** promoter.

DETD	. . . Actin		no	yes	no
RA-3	Actin	no	no	yes	
RA-4	Actin	CME	yes	yes	
RA-5	Actin	CME	yes	no	
RA-6	Actin	CME	no	yes	
RA-7	**CMV**	no	yes	yes	
RA-8	**CMV**	no	yes	no	
RA-9	**CMV**	no	no	yes	
RA-10	**CMV**	CME	yes	yes	
RA-11	**CMV**	CME	yes	no	
RA-12	**CMV**	CME	no	yes	
RA-13	MMTV	no	yes	yes	
RA-14	MMTV	no	yes	no	
RA-15	MMTV	no	no	yes	
RA-16	MMTV	CME	yes	yes	
RA-17	MMTV. . .				

DETD . . . HIV 5' LTR promoter can be deleted and replaced with Moloney virus promoter, MMTV LTR, Actin promoter, myosin promoter and **CMV** promoter.

DETD

Construct	Promoter	poly(A)	Amp^r
LA-1	Moloney	HIV 3'LTR	yes

LA-2	Moloney	SV40	yes
LA-3	Moloney	HIV 3'LTR	no
LA-4	Moloney	5V40	no
LA-5	**CMV**	HIV 3'LTR	yes
LA-6	**CMV**	SV40	yes
LA-7	**CMV**	HIV 3'LTR	no
LA-8	**CMV**	SV40	no
LA-9	MMTV	HIV 3'LTR	yes
LA-10	MMTV	SV40	yes
LA-11	MTTV	HIV 3'LTR	no
LA-12	MTTV	SV40	no
LA-13	HIV 5' LTR	HIV. . .	

DETD . . . the thymidine kinase promoter and polyadenylation site. The HIV env coding region is placed under the regulatory control of the **CMV** promoter and SV40 polyadenylation site. The HIV env coding region was obtained as a 2.3 kb PCR fragment form HIV/3B,. . .

DETD . . . the thymidine kinase promoter and polyadenylation site. The HIV gag/pol coding region is placed under the regulatory control of the **CMV** promoter and SV40 polyadenylation site. The HIV gag/pol coding region was obtained from HIV MN, Genebank sequence MI7449, and includes. . .

DETD . . . the thymidine kinase promoter and polyadenylation site. The HIV gag/pol coding region is placed under the regulatory control of the **CMV** promoter and SV40 polyadenylation site. The HIV gag/pol coding region was obtained from HIV MN, Genebank sequence MI7449, and includes. . .

DETD . . . includes a Kanamycin resistance gene and a pBR322 origin of DNA replication. The sequences provided for transcription regulation include: a **cytomegalovirus** promoter; a Rous sarcoma virus enhancer; and an SV40 polyadenylation signal. The HIV-1 sequences included in pGAGPOL.rev include a sequence. . .

DETD Several safety features are included in pGAGPOL.rev. These include use of the **CMV** promoter and a non-retroviral poly(A) site. Furthermore, deletion of the ψ sequence limits the ability to package viral RNA. In. . .

DETD . . . includes a Kanamycin resistance gene and a pBR322 origin of DNA replication. The sequences provided for transcription regulation include: a **cytomegalovirus** promoter; a Rous sarcoma virus enhancer; and an SV40 polyadenylation signal. The HIV-1 sequences included in pENV include a sequence. . .

DETD Several safety features are included in PGAGPOL.rev. These include use of the **CMV** promoter and a non-retroviral poly(A) site. Furthermore, tat has been deleted and a 50% deletion of nef yields an "inactive". . .

DETD Step 4a. Digest with BamHI and ligate with the **CMV** promoter obtained by PCR of pCEP4 (Invitrogen, San Diego, Calif.) with primers SEQ ID NO.:27 and SEQ ID NO.:28.

DETD . . . be any promoter functional in a human cell. It is preferred that the promoter is an SV40 promoter or a **CMV** promoter, preferably a **CMV immediate early promoter**. The polyadenylation signal may be any polyadenylation signal functional in a human cell. It is preferred that the polyadenylation signal. . .

DETD . . . be any promoter functional in a human cell. It is preferred that the promoter is an SV40 promoter or a **CMV** promoter, preferably a immediate early **CMV** promoter. The polyadenylation signal may be any polyadenylation signal functional in a human cell. It is preferred that the polyadenylation. . .

DETD . . . identical to or substantially similar to epitopes of HIV proteins. The coding sequence is under the regulatory control of the **CMV immediate early promoter** and the SV40 minor polyadenylation signal.

DETD . . . encodes at least one HIV protein or a fragment thereof. The coding sequence is under the regulatory control of the **CMV immediate early promoter** and the SV40 minor polyadenylation signal. The HIV protein is selected from the group consisting of gag, pol, env and. . .

DETD . . . identical to or substantially similar to epitopes of HIV

proteins. The coding sequence is under the regulatory control of the **CMV immediate early promoter** and the SV40 minor polyadenylation signal. The two expression units are encoded in opposite directions of each other.

DETD . . . protein or a fragment thereof. Each expression unit comprises a coding sequence that is under the regulatory control of the **CMV immediate early promoter** and the SV40 minor polyadenylation signal. The HIV protein is selected from the group consisting of gag, pol, env and. . .

DETD . . . examples include Senilis viruses, RossRiver virus and Eastern & Western Equine*** encephalitis. Reovirus: (Medical) Rubella virus.

Flariviridue Family
Examples include: (Medical) **dengue**, yellow fever, Japanese encephalitis, St. Louis encephalitis and tick borne **encephalitis viruses**.

Hepatitis C Virus: (Medical) these viruses are not placed in a family yet but are believed to be either a togavirus or a **flavivirus**. Most similarity is with togavirus family.

Coronavirus Family:
(Medical and Veterinary)
Infectious bronchitis virus (poultry)
Porcine transmissible gastroenteric virus (pig)
Porcine. . . enteritis

Feline panleucopeniavirus
Canine parvovirus
Porcine parvovirus
Herpesvirus Family
Sub-Family: alphaherpesviridue
Genera: Simplexvirus (Medical)
HSVI, HSVII
Varicellovirus: (Medical - Veterinary)
pseudorabies - varicella zoster
Sub-Family - betaherpesviridue
Genera: **Cytomegalovirus** (Medical)
HCMV
Muromegalovirus
Sub-Family: Gammaherpesviridue
Genera: Lymphocryptovirus (Medical)
EBV - (Burkitts lympho)
Rhadinovirus
Poxvirus Family
Sub-Family: Chordopoxviridue (Medical - Veterinary)
Genera: Variola (Smallpox)
Vaccinia (Cowpox)

. . .

CLM What is claimed is:

. . . hepatitis B virus; hepatitis C virus; human papilloma virus, HPV; Herpes simplex 1 virus, HSV1; Herpes simplex 2 virus, HSV2; **Cytomegalovirus, CMV**; Epstein-Barr virus, EBR; rhinovirus; and, coronavirus.

. . . hepatitis B virus; hepatitis C virus; human papilloma virus, HPV; Herpes simplex 1 virus, HSV1; Herpes simplex 2 virus, HSV2; **Cytomegalovirus, CMV**; Epstein-Barr virus, EBR; rhinovirus; and, coronavirus.

L33 ANSWER 6 OF 6 USPATFULL on STN
97:3820 **Genetic immunization**.
Weiner, David B., Merion, PA, United States
Williams, William V., Havertown, PA, United States
Wang, Bin, Havertown, PA, United States

The Wistar Institute, Philadelphia, PA, United States (U.S. corporation); The Trustees of the University of Pennsylvania, Philadelphia, PA, United States (U.S. corporation)
US 5593972 19970114
APPLICATION: US 1993-125012 19930921 (8) <--
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Methods of prophylactic and therapeutic immunization of an individual against pathogen infection, diseases associated with hyperproliferative cells and autoimmune diseases are disclosed. The methods comprise the steps of administering to cells of an individual, a nucleic acid molecule that comprises a nucleotide sequence that encodes a protein which comprises at least one epitope that is identical or substantially similar to an epitope of a pathogen antigen, a hyperproliferative cell associated protein or a protein associated with autoimmune disease respectively. In each case, nucleotide sequence is operably linked to regulatory sequences to enable expression in the cells. The nucleic acid molecule is free of viral particles and capable of being expressed in said cells. The cells may be contacted cells with a cell stimulating agent. Methods of prophylactically and therapeutically immunizing an individual against HIV are disclosed. Pharmaceutical compositions and kits for practicing methods of the present invention are disclosed.
TI **Genetic immunization**
AI US 1993-125012 19930921 (8) <--
DETD . . . cell surface. The Class I MHC-target antigen complexes are capable of stimulating CD8+ T-cells, which are phenotypically the killer/suppressor cells. **Genetic immunization** according to the present invention is thus capable of eliciting cytotoxic T-cell (CTL) responses (killer cell responses). It has been observed that **genetic immunization** according to the present invention is more likely to elicit CTL responses than other methods of immunization.
DETD . . . elimination of deleterious cell types which, during their production of proteins, display antigens bound by Class I MHC molecules. Therefore, **genetic immunization** according to the present invention is more likely to result in anti-pathogen protection and therapy than standard immunization using killed,. . .
DETD **Genetic immunization** according to the present invention elicits an effective immune response without the use of infective agents or infective vectors. Vaccination. . .
DETD . . . the individual. Regulatory elements for DNA include a promoter and a polyadenylation signal. In addition, other elements, such as a **Kozak** region, may also be included in the genetic construct.
DETD . . . Tumor Virus (MMTV) promoter, Human Immunodeficiency Virus (HIV) such as the HIV Long Terminal Repeat (LTR) promoter, Moloney virus, ALV, **Cytomegalovirus** (**CMV**) such as the **CMV immediate early promoter**, Epstein Barr Virus (EBV), Rous Sarcoma Virus (RSV) as well as promoters from human genes such as human Actin, human. . .
DETD . . . but not limited to: human Actin, human Myosin, human Hemoglobin, human muscle creatine and viral enhancers such as those from **CMV**, RSV and EBV.
DETD . . . non-human as well as human individuals against pathogens and protein specific disorders and diseases. Accordingly, the present invention relates to **genetic immunization** of mammals, birds and fish. The methods of the present invention can be particularly useful for mammalian species including human,. . .
DETD . . . individual. The administration protocols and genetic constructs useful in gene therapy applications are the same as those described above for **genetic immunization** except the genetic constructs include nucleotide sequences that encode proteins whose presence in the individual will eliminate a deficiency in. . .
DETD The present invention provides an HIV vaccine using direct **genetic immunization**. Genetic constructs are provided which, when delivered into the cells of an individual, are expressed to produce HIV proteins. According. . .
DETD . . . and a polyadenylation signal. The promoter may be selected from the group consisting of: HIV LTR, human Actin, human Myosin, **CMV**, RSV,

Moloney, HTLV, human Hemoglobin, human muscle creatine and EBV. The enhancer may be selected from the group consisting of: human Actin, human Myosin, **CMV**, RSV, human Hemoglobin, human muscle creatine and EBV. The polyadenylation signal may be selected from the group consisting of: LTR. . .

DETD . . . HXB2 was amplified via PCR and cloned into the expression vector pCNDA/neo (Invitrogen). This plasmid drives envelope production through the **CMV** promoter.

DETD The following is a description of the use of **genetic immunization** for elicitation of an anti-human immunodeficiency virus type 1 (HIV-1) immune response in mice by administering a DNA construct that. . .

DETD In the **genetic immunization** procedure described herein, the quadriceps muscles of BALB/c mice were injected with 100 μl of 0.5% bupivacaine-HCl and 0.1% methylparaben. . .

DETD To determine whether the antisera generated by **DNA immunization** possessed antiviral activity, the ability of the antisera to neutralize HIV-1 infection was examined. Cell-free HIV-1/III_B virus at 100 $TCID_{50}$. . .

DETD . . . Berman P. W., et al., (1990) Nature 345:622-625. The reasons for the more effective generation of anti-viral activities by the **genetic immunization** than by recombinant protein immunization are not clear. However, the differences in the generated immune responses may be due to. . .

DETD . . . isotypes indicates that a secondary immune response has taken place, and further suggests that helper T-cells can be elicited by **genetic immunization.**

DETD In the **genetic immunization** procedure described herein, the quadriceps muscles of BALB/c mice were injected with 100 μl of 0.5% bupivacaine-HCl and 0.1% methylparaben. . .

DETD . . . expression is MMTV LTR. The promoter may be deleted and replaced with Actin promoter, myosin promoter, HIV LTR promoter and **CMV** promoter.

DETD . . . Actin no yes no

RA-3	Actin	no	no	yes
RA-4	Actin	CME	yes	yes
RA-5	Actin	CME	yes	no
RA-6	Actin	CME	no	yes
RA-7	**CMV**	no	yes	yes
RA-8	**CMV**	no	yes	no
RA-9	**CMV**	no	no	yes
RA-10	**CMV**	CME	yes	yes
RA-11	**CMV**	CME	yes	no
RA-12	**CMV**	CME	no	yes
RA-13	MMTV	no	yes	yes
RA-14	MMTV	no	yes	no
RA-15	MMTV	no	no	yes
RA-16	MMTV	CME	yes	yes
RA-17	MMTV. . .			

DETD . . . The HIV 5'LTR promoter can be deleted and replaced with Moloney virus promoter, MMTV LTR, Actin promoter, myosin promoter and **CMV** promoter.

DETD

Construct	Promoter	poly(A)	Amp^r
LA-1	Moloney	HIV 3' LTR	yes
LA-2	Moloney	SV40	yes
LA-3	Moloney	HIV 3' LTR	no
LA-4	Moloney	SV40	no
LA-5	**CMV**	HIV 3' LTR	yes
LA-6	**CMV**	SV40	yes
LA-7	**CMV**	HIV 3' LTR	no
LA-8	**CMV**	SV40	no
LA-9	MMTV	HIV 3' LTR	yes
LA-10	MMTV	SV40	yes
LA-11	MMTV	HIV 3' LTR	no

LA-12 HIV SV40 NO

LA-13 HIV 5'. . .

DETD . . . the thymidine kinase promoter and polyadenylation site. The HIV env coding region is placed under the regulatory control of the **CMV** promoter and SV40 polyadenylation site. The HIV env coding region was obtained as a 2.3 kb PCR fragment form HIV/3B,. . .

DETD . . . the thymidine kinase promoter and polyadenylation site. The HIV gag/pol coding region is placed under the regulatory control of the **CMV** promoter and SV40 polyadenylation site. The HIV gag/pol coding region was obtained from HIV MN, Genebank sequence MI7449, and includes. . .

DETD . . . the thymidine kinase promoter and polyadenylation site. The HIV gag/pol coding region is placed under the regulatory control of the **CMV** promoter and SV40 polyadenylation site. The HIV gag/pol coding region was obtained from HIV MN, Genebank sequence MI7449, and includes. . .

DETD . . . includes a Kanamycin resistance gene and a pBR322 origin of DNA replication. The sequences provided for transcription regulation include: a **cytomegalovirus** promoter; a Rous sarcoma virus enhancer; and an SV40 polyadenylation signal. The HIV-1 sequences included in pGAGPOL.rev include a sequence. . .

DETD Several safety features are included in pGAGPOL.rev. These include use of the **CMV** promoter and a non-retroviral poly(A) site. Furthermore, deletion of the ψ sequence limits the ability to package viral RNA. In. . .

DETD . . . includes a Kanamycin resistance gene and a pBR322 origin of DNA replication. The sequences provided for transcription regulation include: a **cytomegalovirus** promoter; a Rous sarcoma virus enhancer; and an SV40 polyadenylation signal. The HIV-1 sequences included in pENV include a sequence. . .

DETD Several safety features are included in pGAGPOL.rev. These include use of the **CMV** promoter and a non-retroviral poly(A) site. Furthermore, tat has been deleted and a 50% deletion of nef yields an "inactive". . .

DETD Step 4a. Digest with BamHI and ligate with the **CMV** promoter obtained by PCR of pCEP4 (Invitrogen, San Diego Calif.) with primers SEQ ID NO.:27 and SEQ ID NO.:28.

DETD . . . be any promoter functional in a human cell. It is preferred that the promoter is an SV40 promoter or a **CMV** promoter, preferably a **CMV immediate early promoter**. The polyadenylation signal may be any polyadenylation signal functional in a human cell. It is preferred that the polyadenylation signal. . .

DETD . . . be any promoter functional in a human cell. It is preferred that the promoter is an SV40 promoter or a **CMV** promoter, preferably a immediate early **CMV** promoter. The polyadenylation signal may be any polyadenylation signal functional in a human cell. It is preferred that the polyadenylation. . .

DETD . . . identical to or substantially similar to epitopes of HIV proteins. The coding sequence is under the regulatory control of the **CMV immediate early promoter** and the SV40 minor polyadenylation signal.

DETD . . . encodes at least one HIV protein or a fragment thereof. The coding sequence is under the regulatory control of the **CMV immediate early promoter** and the SV40 minor polyadenylation signal. The HIV protein is selected from the group consisting of gag, pol, env and. . .

DETD . . . identical to or substantially similar to epitopes of HIV proteins. The coding sequence is under the regulatory control of the **CMV immediate early promoter** and the SV40 minor polyadenylation signal. The two expression units are encoded in opposite directions of each other.

DETD . . . protein or a fragment thereof. Each expression unit comprises a coding sequence that is under the regulatory control of the **CMV immediate early promoter** and the SV40 minor polyadenylation signal. The HIV protein is selected from the group consisting of gag, pol, env and. . .

DETD Examples include: (Medical) **dengue**, yellow fever, Japanese

encephalitis, St. Louis encephalitis and tick borne **encephalitis viruses**.

DETD . . . (Medical) these viruses are not placed in a family yet but are believed to be either a togavirus or a **flavivirus**. Most similarity is with togavirus family.

DETD **Cytomegalovirus** (Medical)

CLM What is claimed is:

. . . hepatitis B virus; hepatitis C virus; human papilloma virus, HPV; Herpes simplex 1 virus, HSV1; Herpes simplex 2 virus, HSV2; **Cytomegalovirus**, **CMV**; Epstein-Barr virus, EBR; rhinovirus; and, coronavirus.

=> d his

(FILE 'HOME' ENTERED AT 15:58:13 ON 30 APR 2004)

FILE 'USPATFULL' ENTERED AT 15:59:07 ON 30 APR 2004
E CHANG GWONG JEN/IN
L1 1 S E4

FILE 'WPIDS' ENTERED AT 16:00:09 ON 30 APR 2004
E CHANG G J/IN
L2 106 S E3
L3 2 S L2 AND FLAVIVIR?

FILE 'MEDLINE' ENTERED AT 16:01:13 ON 30 APR 2004
E CHANG G J/AU
L4 49 S E3
L5 29 S L4 AND (FLAVIVIR? OR YELLOW OR DENGUE OR ENCEPHALITIS)
L6 9 S L5 AND (PRM OR E OR PRM/M OR PRM/E)

FILE 'USPATFULL' ENTERED AT 16:06:37 ON 30 APR 2004
L7 3470 S (FLAVIVIR? OR YELLOW FEVER VIR? OR DENGUE OR ENCEPHALITIS VIR
L8 79 S L7 AND (PRM OR PREMEMBRANE)
L9 79 S L8 AND (E OR ENVELOPE)
L10 79 S L9 AND (M OR MEMBRANE)
L11 43 S L10 AND (SIGNAL SEQUENCE)
L12 5 S L11 AND KOZAK
L13 38 S L11 NOT L12
L14 15 S L13 AND AY<1999
L15 805 S L7 AND (KOZAK OR RIBOSOME BINDING SITE)
L16 2 S L15 AND KOZAK/CLM
L17 0 S L15 AND (KOZAK RIBOSOME BINDING SITE? OR KOZAK CONCENSUS SEQU
L18 716 S L15 AND (CMV OR CYTOMEGALOVIRUS)
L19 191 S L18 AND (IE OR IMMEDIATE-EARLY PROMOTER?)
L20 30 SS L19 AND (CMV/CLM OR CYTOMEGALOVIRUS/CLM)
L21 2 S L20 AND (IE/CLM OR IMMEDIATE-EARLY/CLM)
L22 28 S L20 NOT L21
L23 10 S L20 AND AY<1999
L24 743 S L15 AND (POLYADENYLATION OR POLY-A)
L25 423 S L24 AND (POLYADENYLATION (5W) TERMINAT?)
L26 2 S L25 AND L23
E KONISHI E/IN
L27 1 S E4
E KOZAK M/IN
L28 1 S E5

FILE 'MEDLINE' ENTERED AT 16:45:49 ON 30 APR 2004
E KONISHI E/AU
L29 102 S E3-E5
L30 17 S L29 AND (PRM OR PREMEMBRANE)
L31 17 S L30 AND (E OR ENVELOPE OR ENV)

FILE 'USPATFULL' ENTERED AT 16:53:49 ON 30 APR 2004

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L32           400 S L15 AND (DNA VACCIN? OR GENETIC IMMUNIZAT? OR DNA IMMUNIZ?)
L33             6 S L32 AND L23

=>
=> file medline
COST IN U.S. DOLLARS                                    SINCE FILE      TOTAL
                                                           ENTRY    SESSION
FULL ESTIMATED COST                                        21.84     246.01

FILE 'MEDLINE' ENTERED AT 16:58:36 ON 30 APR 2004

 FILE LAST UPDATED: 29 APR 2004 (20040429/UP).  FILE COVERS 1951 TO DATE.

 On February 29, 2004, the 2004 MeSH terms were loaded.  See HELP RLOAD
 for details. OLDMEDLINE now back to 1951.

 MEDLINE thesauri in the /CN, /CT, and /MN fields incorporate the
 MeSH 2004 vocabulary.  See http://www.nlm.nih.gov/mesh/ and
 http://www.nlm.nih.gov/pubs/techbull/nd03/nd03_mesh.html for a
 description of changes.

 This file contains CAS Registry Numbers for easy and accurate
 substance identification.

=> d his

     (FILE 'HOME' ENTERED AT 15:58:13 ON 30 APR 2004)

     FILE 'USPATFULL' ENTERED AT 15:59:07 ON 30 APR 2004
                E CHANG GWONG JEN/IN
L1              1 S E4

     FILE 'WPIDS' ENTERED AT 16:00:09 ON 30 APR 2004
                E CHANG G J/IN
L2            106 S E3
L3              2 S L2 AND FLAVIVIR?

     FILE 'MEDLINE' ENTERED AT 16:01:13 ON 30 APR 2004
                E CHANG G J/AU
L4             49 S E3
L5             29 S L4 AND (FLAVIVIR? OR YELLOW OR DENGUE OR ENCEPHALITIS)
L6              9 S L5 AND (PRM OR E OR PRM/M OR PRM/E)

     FILE 'USPATFULL' ENTERED AT 16:06:37 ON 30 APR 2004
L7           3470 S (FLAVIVIR? OR YELLOW FEVER VIR? OR DENGUE OR ENCEPHALITIS VIR
L8             79 S L7 AND (PRM OR PREMEMBRANE)
L9             79 S L8 AND (E OR ENVELOPE)
L10            79 S L9 AND (M OR MEMBRANE)
L11            43 S L10 AND (SIGNAL SEQUENCE)
L12             5 S L11 AND KOZAK
L13            38 S L11 NOT L12
L14            15 S L13 AND AY<1999
L15           805 S L7 AND (KOZAK OR RIBOSOME BINDING SITE)
L16             2 S L15 AND KOZAK/CLM
L17             0 S L15 AND (KOZAK RIBOSOME BINDING SITE? OR KOZAK CONCENSUS SEQU
L18           716 S L15 AND (CMV OR CYTOMEGALOVIRUS)
L19           191 S L18 AND (IE OR IMMEDIATE-EARLY PROMOTER?)
L20            30 SS L19 AND (CMV/CLM OR CYTOMEGALOVIRUS/CLM)
L21             2 S L20 AND (IE/CLM OR IMMEDIATE-EARLY/CLM)
L22            28 S L20 NOT L21
L23            10 S L20 AND AY<1999
L24           743 S L15 AND (POLYADENYLATION OR POLY-A)
L25           423 S L24 AND (POLYADENYLATION (5W) TERMINAT?)
L26             2 S L25 AND L23
                E KONISHI E/IN
L27             1 S E4
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                E KOZAK M/AU
L28             1 S E5

     FILE 'MEDLINE' ENTERED AT 16:45:49 ON 30 APR 2004
                E KONISHI E/AU
L29           102 S E3-E5
L30            17 S L29 AND (PRM OR PREMEMBRANE)
L31            17 S L30 AND (E OR ENVELOPE OR ENV)

     FILE 'USPATFULL' ENTERED AT 16:53:49 ON 30 APR 2004
L32           460 S L15 AND (DNA VACCIN? OR GENETIC IMMUNIZAT? OR DNA IMMUNIZ?)
L33             6 S L32 AND L23

     FILE 'MEDLINE' ENTERED AT 16:58:36 ON 30 APR 2004

=> s (flavivir? or yellow fever vir? or dengue or encephalitis vir?)
          3172 FLAVIVIR?
         18667 YELLOW
        102209 FEVER
        624737 VIR?
           865 YELLOW FEVER VIR?
                 (YELLOW(W)FEVER(W)VIR?)
          4267 DENGUE
         26248 ENCEPHALITIS
        624737 VIR?
          8314 ENCEPHALITIS VIR?
                 (ENCEPHALITIS(W)VIR?)
L34      14853 (FLAVIVIR? OR YELLOW FEVER VIR? OR DENGUE OR ENCEPHALITIS VIR?)

=> s l34 and (prM or premembrane)
           544 PRM
            87 PREMEMBRANE
L35         227 L34 AND (PRM OR PREMEMBRANE)

=> s l35 and (M or membrane)
        376250 M
        572801 MEMBRANE
L36         135 L35 AND (M OR MEMBRANE)

=> s l36 and (E or envelope)
        628030 E
         34155 ENVELOPE
L37         127 L36 AND (E OR ENVELOPE)

=> s l37 and (DNA vaccin? or genetic immunization or DNA immunization or polynucleotide vaccin?
        741015 DNA
        146418 VACCIN?
          2147 DNA VACCIN?
                 (DNA(W)VACCIN?)
        485047 GENETIC
         85706 IMMUNIZATION
           247 GENETIC IMMUNIZATION
                 (GENETIC(W)IMMUNIZATION)
        741015 DNA
         85706 IMMUNIZATION
           537 DNA IMMUNIZATION
                 (DNA(W)IMMUNIZATION)
          3980 POLYNUCLEOTIDE
        146418 VACCIN?
            24 POLYNUCLEOTIDE VACCIN?
                 (POLYNUCLEOTIDE(W)VACCIN?)
L38          15 L37 AND (DNA VACCIN? OR GENETIC IMMUNIZATION OR DNA IMMUNIZATION
                 OR POLYNUCLEOTIDE VACCIN?)

=> s l38 and py<1999
      11862550 PY<1999
```

L39 4 L38 AND PY<1999

=> d l39,cbib,ab,1-4

L39 ANSWER 1 OF 4 MEDLINE on STN
1998445455. PubMed ID: 9770429. DNA-based and alphavirus-vectored immunisation with **prM** and **E** proteins elicits long-lived and protective immunity against the **flavivirus**, Murray Valley **encephalitis virus**. Colombage G; Hall R; Pavy M; Lobigs M. (John Curtin School of Medical Research, The Australian National University, Canberra, ACT, 2601, Australia.) Virology, **(1998 Oct 10)** 250 (1) 151-63. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.
AB The immunogenicity and protective efficacy of DNA-based vaccination with plasmids encoding the **membrane** proteins **prM** and **E** of the **flavivirus** Murray Valley **encephalitis virus** (MVE) were investigated. Gene gun-mediated intradermal delivery of DNA encoding the **prM** and **E** proteins elicited long-lived, virus-neutralising antibody responses in three inbred strains of mice and provided protection from challenge with a high titer inoculum of MVE. Intramuscular **DNA vaccination** by needle injection also induced MVE-specific antibodies that conferred resistance to challenge with live virus but failed to reduce virus infectivity in vitro. The two routes of DNA-based vaccination with **prM** and **E** encoding plasmids resulted in humoral immunty with distinct IgG subtypes. MVE-specific IgG1 antibodies were always prevalent after intradermal **DNA vaccination** via a gene gun but not detected when mice were immunised with DNA by the intramuscular route or infected with live virus. We also tested a Semliki Forest virus replicon as vector for a **flavivirus prM** and **E** protein-based subunit vaccine. Single-cycle infections in mice vaccinated with packaged recombinant replicon particles elicited durable, MVE-specific, and virus-neutralising antibody responses.

L39 ANSWER 2 OF 4 MEDLINE on STN
1998080404. PubMed ID: 9420215. **DNA immunization** with Japanese **encephalitis virus** nonstructural protein NS1 elicits protective immunity in mice. Lin Y L; Chen L K; Liao C L; Yeh C T; Ma S H; Chen J L; Huang Y L; Chen S S; Chiang H Y. (Institute of Preventive Medicine, National Defense Medical Center, Taipei, Taiwan, Republic of China.. yll@msl1.hinet.net) . Journal of virology, **(1998 Jan)** 72 (1) 191-200. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.
AB Japanese **encephalitis virus** (JEV), a mosquito-borne **flavivirus**, is a zoonotic pathogen that is prevalent in some Southeast Asian countries and causes acute encephalitis in humans. To evaluate the potential application of gene immunization to JEV infection, we characterized the immune responses from mice intramuscularly injected with plasmid DNA encoding JEV glycoproteins, including the precursor **membrane** (**prM**) plus **envelope** (**E**) proteins and the nonstructural protein NS1. When injected with the plasmid expressing **prM** plus **E**, 70% of the immunized mice survived after a lethal JEV challenge, whereas when immunized with the plasmid expressing NS1, 90% of the mice survived after a lethal challenge. As a control, the mice immunized with the DNA vector pcDNA3 showed a low level (40%) of protection, suggesting a nonspecific adjuvant effect of the plasmid DNA. Despite having no detectable neutralizing activity, the NS1 immunization elicited a strong antibody response exhibiting cytolytic activity against JEV-infected cells in a complement-dependent manner. By contrast, immunization with a construct expressing a longer NS1 protein (NS1'), containing an extra 60-amino-acid portion from the N terminus of NS2A, failed to protect mice against a lethal challenge. Biochemical analyses revealed that when individually expressed, NS1 but not NS1' could be readily secreted as a homodimer in large quantity and could also be efficiently expressed on the cell surface. Interestingly, when NS1 and NS1' coexisted in cells, the level of NS1 cell surface expression was much lower than that in cells expressing NS1 alone. These data imply that the presence of partial NS2A might have a negative influence on an NS1-based **DNA vaccine**. The

results herein clearly illustrate that immunization with DNA expressing NS1 alone is sufficient to protect mice against a lethal JEV challenge.

L39 ANSWER 3 OF 4 MEDLINE on STN

1998037671. PubMed ID: 9371620. Naked **DNA vaccines** expressing the **prM** and **E** genes of Russian spring summer **encephalitis virus** and Central European **encephalitis virus** protect mice from homologous and heterologous challenge. Schmaljohn C; Vanderzanden L; Bray M; Custer D; Meyer B; Li D; Rossi C; Fuller D; Fuller J; Haynes J; Huggins J. (Virology Division, United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Maryland 21702-5011, USA.) Journal of virology, **(1997 Dec)** 71 (12) 9563-9. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Naked **DNA vaccines** expressing the **prM** and **E** genes of two tick-borne **flaviviruses**, Russian spring summer encephalitis (RSSE) virus and Central European encephalitis (CEE) virus were evaluated in mice. The vaccines were administered by particle bombardment of DNA-coated gold beads by Accell gene gun inoculation. Two immunizations of 0.5 to 1 microg of RSSE or CEE constructs/dose, delivered at 4-week intervals, elicited cross-reactive antibodies detectable by enzyme-linked immunosorbent assay and high-titer neutralizing antibodies to CEE virus. Cross-challenge experiments demonstrated that either vaccine induced protective immunity to homologous or heterologous RSSE or CEE virus challenge. The absence of antibody titer increases after challenge and the presence of antibodies to **E** and **prM**, but not NS1, both before and after challenge suggest that the vaccines prevented productive replication of the challenge virus. One vaccination with 0.5 microg of CEE virus DNA provided protective immunity for at least 2 months, and two vaccinations protected mice from challenge with CEE virus for at least 6 months.

L39 ANSWER 4 OF 4 MEDLINE on STN

96215657. PubMed ID: 8645110. Immunisation with DNA polynucleotides protects mice against lethal challenge with St. Louis **encephalitis virus**. Phillpotts R J; Venugopal K; Brooks T. (Microbiology Group, Chemical and Biological Defence Establishment, Porton Down, Wiltshire, U.K.) Archives of virology, **(1996)** 141 (3-4) 743-9. Journal code: 7506870. ISSN: 0304-8608. Pub. country: Austria. Language: English.

AB In vivo transfection by intramuscular injection with plasmids expressing the immunogenic proteins of microbial pathogens has considerable potential as a vaccination strategy against many pathogens of both man and animals. Here we report that weanling mice given a single intramuscular injection of 50 micrograms of a plasmid, pSLE1 expressing the St. Louis **encephalitis virus** (SLE) **prM/E** protein under the control of the cytomegalovirus immediate early protein promoter produced SLE-specific antibody and were protected against lethal challenge with the virulent virus. **Polynucleotide vaccine** technology provides a unique opportunity to produce vaccines against **flavivirus** diseases of low incidence cheaply and rapidly, and to produce multivalent vaccines such as would be required for immunisation against **dengue** virus disease.

=> d his

(FILE 'HOME' ENTERED AT 15:58:13 ON 30 APR 2004)

FILE 'USPATFULL' ENTERED AT 15:59:07 ON 30 APR 2004
E CHANG GWONG JEN/IN
L1 1 S E4

FILE 'WPIDS' ENTERED AT 16:00:09 ON 30 APR 2004
E CHANG G J/IN
L2 106 S E3
L3 2 S L2 AND FLAVIVIR?

FILE 'MEDLINE' ENTERED AT 16:01:13 ON 30 APR 2004
E CHANG G J/AU

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L4            45 S E3
L5            29 S L4 AND (FLAVIVIR? OR YELLOW OR DENGUE OR ENCEPHALITIS)
L6             9 S L5 AND (PRM OR E OR PRM/M OR PRM/E)

     FILE 'USPATFULL' ENTERED AT 16:06:37 ON 30 APR 2004
L7          3470 S (FLAVIVIR? OR YELLOW FEVER VIR? OR DENGUE OR ENCEPHALITIS VIR
L8            79 S L7 AND (PRM OR PREMEMBRANE)
L9            79 S L8 AND (E OR ENVELOPE)
L10           79 S L9 AND (M OR MEMBRANE)
L11           43 S L10 AND (SIGNAL SEQUENCE)
L12            5 S L11 AND KOZAK
L13           38 S L11 NOT L12
L14           15 S L13 AND AY<1999
L15          805 S L7 AND (KOZAK OR RIBOSOME BINDING SITE)
L16            2 S L15 AND KOZAK/CLM
L17            0 S L15 AND (KOZAK RIBOSOME BINDING SITE? OR KOZAK CONCENSUS SEQU
L18          716 S L15 AND (CMV OR CYTOMEGALOVIRUS)
L19          191 S L18 AND (IE OR IMMEDIATE-EARLY PROMOTER?)
L20           30 SS L19 AND (CMV/CLM OR CYTOMEGALOVIRUS/CLM)
L21            2 S L20 AND (IE/CLM OR IMMEDIATE-EARLY/CLM)
L22           28 S L20 NOT L21
L23           10 S L20 AND AY<1999
L24          743 S L15 AND (POLYADENYLATION OR POLY-A)
L25          423 S L24 AND (POLYADENYLATION (5W) TERMINAT?)
L26            2 S L25 AND L23
                 E KONISHI E/IN
L27            1 S E4
                 E KOZAK M/IN
L28            1 S E5

     FILE 'MEDLINE' ENTERED AT 16:45:49 ON 30 APR 2004
                 E KONISHI E/AU
L29          102 S E3-E5
L30           17 S L29 AND (PRM OR PREMEMBRANE)
L31           17 S L30 AND (E OR ENVELOPE OR ENV)

     FILE 'USPATFULL' ENTERED AT 16:53:49 ON 30 APR 2004
L32          460 S L15 AND (DNA VACCIN? OR GENETIC IMMUNIZAT? OR DNA IMMUNIZ?)
L33            6 S L32 AND L23

     FILE 'MEDLINE' ENTERED AT 16:58:36 ON 30 APR 2004
L34        14853 S (FLAVIVIR? OR YELLOW FEVER VIR? OR DENGUE OR ENCEPHALITIS VIR
L35          227 S L34 AND (PRM OR PREMEMBRANE)
L36          135 S L35 AND (M OR MEMBRANE)
L37          127 S L36 AND (E OR ENVELOPE)
L38           15 S L37 AND (DNA VACCIN? OR GENETIC IMMUNIZATION OR DNA IMMUNIZAT
L39            4 S L38 AND PY<1999

=> log off
ALL L# QUERIES AND ANSWER SETS ARE DELETED AT LOGOFF
LOGOFF? (Y)/N/HOLD:y
STN INTERNATIONAL LOGOFF AT 17:04:30 ON 30 APR 2004
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